ECOLOGICAL AND EVOLUTIONARY EFFECTS OF DISPERSAL ON FRESHWATER ZOOPLANKTON

BY

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DISSERTATION

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Abstract

A recent focus on contemporary evolution and the connections between communities has sought to more closely integrate the fields of ecology and evolutionary biology. Studies of coevolutionary dynamics, life history evolution, and rapid local adaptation demonstrate that ecological circumstances can dictate evolutionary trajectories. Thus, variation in species identity, trait distributions, and genetic composition may be maintained among ecologically divergent habitats. New theories and hypotheses (e.g., metacommunity theory and the Monopolization hypothesis) have been developed to understand better the processes occurring in spatially structured environments and how the movement of individuals among habitats contributes to ecology and evolution at broader scales. As few empirical studies of these theories exist, this work seeks to further test these concepts.

Spatial and temporal dispersal are the mechanisms that connect habitats to one another. Both processes allow organisms to leave conditions that are suboptimal or unfavorable, and enable colonization and invasion, species range expansion, and gene flow among populations. Freshwater zooplankton are aquatic crustaceans that typically develop resting stages as part of their life cycle. Their dormant propagules allow organisms to disperse both temporally and among habitats. Additionally, because a number of species are cyclically parthenogenetic, they make excellent model organisms for studying evolutionary questions in a controlled environment.

Here, I use freshwater zooplankton communities as model systems to explore the mechanisms and consequences of dispersal and to test these nascent theories on the influence of spatial structure in natural systems. In Chapter one, I use field experiments and mathematical models to determine the range of adult zooplankton dispersal over land and what vectors are moving zooplankton. Chapter two focuses on prolonged dormancy of one aquatic zooplankter, *Daphnia pulex.* Using statistical models with field and mesocosm experiments, I show that variation in *Daphnia* dormant egg hatching is substantial among populations in nature, and some of that variation can be attributed to genetic differences among the populations. Chapters three and four explore the consequences of dispersal at multiple levels of biological organization. Chapter three seeks to understand the population level consequences of dispersal over evolutionary

time on current patterns of population genetic differentiation. Nearby populations of *D. pulex* often exhibit high population genetic differentiation characteristic of very low dispersal. I explore two alternative hypotheses that seek to explain this pattern. Finally, chapter four is a case study of how dispersal has influenced patterns of variation at the community, trait and genetic levels of biodiversity in a lake metacommunity.

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Chapter 1: Measuring and modeling dispersal of adult zooplankton[1]

1.1 ABSTRACT

Habitat fragmentation poses an inherent problem for metacommunity dynamics, as dispersal among communities is hindered by increasing isolation and the loss of patches. Wetlands are one such system that have undergone excessive destruction and fragmentation in recent years. Zooplankton within these communities have historically been considered frequent and widespread dispersers, but direct, quantitative measures of zooplankton dispersal are rare. In this study, I performed two experiments to quantify zooplankton dispersal and to identify the primary dispersal vectors. I first set up an array of traps at 10, 30, 60, 120 and 180 m around an isolated pond to collect dispersing individuals. Nearly 1500 adult zooplankton were captured in traps up to 180 m from the pond, with approximately 60% of dispersers being captured in traps at 10 m from the pond. A second experiment using open and animal-excluded traps suggested that large animals were the primary dispersal vector for these zooplankton. Using a subset of these data, I fit four models to describe the shape and magnitude of adult cladoceran dispersal at this site. All models showed the majority of cladocerans were deposited very close to the source pond, with three models suggesting the trapping area encompassed 67% or more of the dispersal distances. These results suggest adult zooplankton movement among ponds may be significant in areas where aquatic habitats are plentiful. Yet, in recent years climate change and anthropogenic disturbances have reduced the number and size of aquatic habitats in many regions of the world, likely curtailing effective transport of individuals in many cases. As a result, fragmented zooplankton metacommunities may experience increased dispersal limitation, stronger priority effects, higher levels of inbreeding and selection against traits engendering high dispersability.

1.2 INTRODUCTION

Anthropogenic habitat fragmentation has been long-recognized as a major threat to biodiversity (Saunders et al. 1991, Debinski and Holt 2000). Early work suggested the

[1] The original publication is available at www.springerlink.com or http://dx.doi.org/10.1007/s00442-007-0704-4.

loss of available space would negatively affect both species richness and abundance though mechanisms such as the conversion of continuous habitat to edge habitat, the reduction in area below the minimum home range of organisms, microsite changes and the isolation of the remnant fragments (MacArthur and Wilson 1967, Diamond 1975). While these changes in habitat structure have immediate effects on population survival and subsequently, community diversity, the exchange of individuals among patches may also contribute to the persistence of species in fragmented landscapes. Isolated fragments can be viewed as a set of communities connected by dispersal – or a metacommunity (Gilpin and Hanski 1991, Leibold and Miller 2004). In this context, dispersal influences range expansion rates and gene flow among populations, drives source-sink dynamics, influences local community composition and affects colonization and community assembly dynamics in new and intact habitats (Pulliam 1988, Drake 1991, Bilton et al. 2001, Couvet 2002, Gomez et al. 2002, Bohonak and Jenkins 2003). Lacking dispersal, populations are prone to catastrophic extinctions and genetic consequences such as genetic drift and inbreeding depression (Couvet 2002).

Freshwater zooplankton communities are an excellent system to study how metacommunity structure may be affected by habitat fragmentation. Freshwater ponds are inherently isolated, seemingly closed systems in a matrix of land. Yet, their isolation is not absolute, as individuals have been shown to move between ponds via both direct connections (e.g., rivulets or flooding) (e.g., Michels et al. 2001) and overland dispersal (Cáceres and Soluk 2002, Cohen and Shurin 2003), thereby connecting populations within the metacommunity. Unfortunately, fragmentation has posed an increasing problem for wetland communities. For example, recent land management practices (e.g., agricultural conversion, road building) have resulted in a 53% reduction in the coverage of wetlands in the continental United States (Dahl 1990). The problem has become especially acute in central Illinois, where as much as 90% of original wetlands have been lost in the last 150 years (Suloway and Hubbell 1994). These losses are intensifying differences among communities in their relative isolations, and thus providing an interesting model system to explore fragmentation and metacommunity dynamics.

Dispersal of zooplankton has typically been considered “frequent and widespread” across the landscape with many species classified as cosmopolitan (Brooks

and Dodson 1965, Pennak 1989). Unfortunately, little is known about true dispersal rates in the zooplankton, as tracking movement is challenging (Bilton et al. 2001). While some suggest zooplankton may disperse readily (Cohen and Shurin 2003, Louette and De Meester 2004, 2005), others suggest the opposite (Jenkins 1995, Jenkins and Buikema 1998, Bohonak and Jenkins 2003). Many studies have provided indirect measures of dispersal via gene flow estimates (Boileau et al. 1992), modeling invasions (Havel et al. 2002) or colonization experiments (Jenkins 1995, Jenkins and Buikema 1998, Cáceres and Soluk 2002, Cohen and Shurin 2003, Louette and De Meester 2004, 2005). However, to better understand actual movement patterns, a direct, quantitative measure of zooplankton dispersal is needed.

Additionally, most evidence for the modes of zooplankton dispersal is merely anecdotal. Pioneering dispersal observations date back to Darwin's *On the Origin of Species* (1859), where duck's feet were noted as a method for the transport of mollusks. Since that time, ecologists have identified three main vectors that may disperse zooplankton: wind and rain (Jenkins and Underwood 1998, Brendonck and Riddoch 1999, Cáceres and Soluk 2002, Cohen and Shurin 2003), and animals (Maguire 1963, Proctor 1965, Figuerola et al. 2003, Louette and De Meester 2004). However, with metacommunity fragmentation and local isolation, the relative influence of these vectors on dispersal might change. Cáceres and Soluk (2002) and Cohen and Shurin (2003) examined these dispersal vectors on a local scale, carrying out colonization experiments in open fields near large permanent ponds. They concluded that wind and rain were likely the primary drivers of dispersal at a local scale, rather than animal vectors. Here, I examine dispersal in an alternative setting that is more typical for many natural zooplankton assemblages, an isolated, temporary pond located in a woodlot. Specifically, in this study, I address three questions: 1) What is the magnitude and distribution of local zooplankton movement, and is it similar for all zooplankton taxa? 2) Are local scale zooplankton movements driven by animal vectors or by wind and rain? 3) What implications do these results have for the dispersal of zooplankton in highly fragmented communities?

1.3 METHODS

1.3.1 Study Site

I examined dispersal of zooplankton communities at Trelease Woods, a five hectare woodlot near Urbana, Illinois. Center Pond, the focal pond, is located approximately 60 m from the east edge of the woods. Constructed more than 60 years ago, it has a maximum area and depth of 478 m^2 and 1.26 m, respectively, and typically holds water from December through July. Resident dispersal vectors include deer, raccoons, opossum, squirrels, waterfowl, birds and insects. There is one other pond onsite, located ~400 m away. No other ponds are within 1 km.

1.3.2 Center Pond Zooplankton Counts

To estimate species composition and abundance within Center Pond, I collected samples from April through pond drying (August 2004). I took two water column samples each week (15 to 24 L each) from at least five locations using a 3 L graduated pitcher. Samples were filtered through a 70 μm sieve and stored in >70% ethanol. To estimate the total abundance of zooplankton, I examined three subsamples (or at least 100 individuals per taxa) from each sample. Samples were identified to the family or genus level following Pennak (1989).

To estimate total individuals in the pond, accurate pond volume measurements were required. I created bathymetric and basin surface maps using 172 depth measurements taken throughout the pond at its highest water phase. Using ArcGIS Desktop 9 (Redmond, WA), an inverse multiquadric spline was fit to the depth data. This model minimized the root mean square error for the prediction surface and provided an appropriate surface model for the basin. Then, surface area and volume estimates for the pond were calculated in ArcScene 3D imaging software from water depth estimates. This depth model was combined with species density data to estimate the daily abundance of each taxon.

1.3.3 Field Experiments

To examine zooplankton dispersal near Center Pond, 32 traps were set up to capture zooplankton leaving the pond. Traps were set out along transects in the eight cardinal directions at each of three distances from the pond (10, 30, and 60 m). Four additional traps were placed along the north and south transects at 120 m and 180 m from

the pond. Each trap consisted of a 15 L bucket filled with 13 L of filtered well water (5 μm). Each trap was anchored to the ground to prevent tipping. I also placed four traps, filled with water but closed to dispersal, at 30 m to serve as controls. After nine days, all zooplankton captured in each bucket were collected by filtering the water through a 70 μm sieve; samples were stored in >70% ethanol for later processing. For each bucket, the remaining filtered water was transferred into an ethanol washed 15 L bucket and sterilized with 3.5 ml of 6% bleach solution. After two days, the water was dechlorinated using 1.4 ml of Amquel Plus (Novalek, Hayward, CA) for use in subsequent experimental replicates. The experiment was replicated five times over consecutive two-week periods between 7 April and 10 June 2004 to examine dispersal over potential temporal variation in propagule densities.

To test the assumption of equal dispersal ability among different zooplankton taxa, I calculated the relative frequency of dispersal of each species as the total number of dispersers to all traps divided by the average species density in the pond during a given replicate. Differences in relative frequency among taxa were arcsine-square root transformed to normalize the residuals and tested via one way ANOVA. Additionally, I tested the assumption that reproduction was not occurring within traps (see Appendix A).

In May 2005, I performed a second experiment to assess the importance of animal versus wind and rain dispersal. Wire exclosures (3.5 cm holes, 1 m tall) were placed around dispersal traps at 10 m from the pond to exclude all mammals and birds. Open traps were placed at 30 m. After nine days, traps were emptied as above, and zooplankton were stored in >70% ethanol. For both experiments, I counted all individuals in trap samples to the family or genus level (Pennak 1989).

1.3.4 Model Design

To examine the scale and magnitude of local zooplankton movement, I modeled dispersal using empirical curve fitting techniques common in the terrestrial literature. While mechanistic models are often preferred for describing organismal movement, empirical models are appropriate here, since only understanding the magnitude and scope of dispersal are necessary to address my objectives. To adequately describe these dispersal data with empirical models, I assumed trap counts were time integrated and tested to assure there was no directional bias (or drift) in the dispersal data (see Appendix

B and Turchin and Thoeny 1993). I chose four frequently employed empirical models with different behaviors at the head, center and tail of the dispersal kernel to fit the dispersal data (Table B.1). The inverse power (IP) model is the most leptokurtic of the four models, while the negative exponential (NE) model is the most platykurtic. The mechanistic negative exponential model (MNE), proposed by Turchin and Thoeny (1993), is a variant of the typical negative exponential model that is derived from diffusion principles. The Students' two-dimensional t model (2Dt) was developed by Clark et al. (1999) as a mixture of convex head and fat tailed models. Each of these models assumes a constant source of propagules among replicates. As the pond density changed between sampling events, I included it as a covariate in the model formula to control for changing initial dispersal source size among replicates. The NE, MNE and IP models were fit to the count data by maximum likelihood estimation of generalized linear models assuming a Poisson error structure and a log link function. The log pond density (*D*) was treated as an offset variable for the NE and IP models. For the MNE model, log(D) minus ½ the radial distance (r) served as the offset variable. The models were fit using the 'glm' procedure in R 2.3.1 (R Development Core Team 2006). The 2Dt model was fit via maximum likelihood estimation using an algorithm that minimized the negative log-likelihood of the model ('optim' in R). Again, a Poisson error structure was assumed. To choose among models, I calculated the AIC and ΔAIC values for each of the models and selected those models with the lowest AIC and $\Delta AIC < 2$ (Burnham and Anderson 2002). Using the best fit model and its parameters, I derived two statistics to describe dispersal at this spatial scale: the distance to which a fraction of the dispersers traveled and the percent of pond organisms dispersing daily. Detailed descriptions of these methods and assumptions can be found in Appendix B.

1.4 RESULTS

1.4.1 Field Experiments

A total of 1470 adult zooplankton were trapped over the course of the first experiment, including 615 cladocerans, 247 copepods and 608 rotifers. Six cladoceran, three copepod and seven rotifer taxa were observed (Appendix C). Additionally, 215 cladoceran ephippia were trapped. No zooplankton were found in any of the control traps.

Similar to other dispersal experiments (e.g., Bullock and Clark (2000)), the great majority of individuals were trapped very close to the propagule source. Sixty-two percent of cladocerans, for example, were trapped at 10 m from the pond while only 8% reached 60 m or further. Cladoceran ephippia exhibited similar dispersal ability as 70% were trapped at 10 m while 5% were collected beyond the 30 m traps. Copepods tended to disperse a bit further (40% at 10 m, 29% 60 m or further), although the total number of copepods trapped was much lower. The majority of the rotifers captured were Bdelloids. As Bdelloids likely reproduced during the nine days in the traps, they were excluded from further analyses. Other rotifer abundances were so low that further analyses could not be performed.

Dispersal patterns were not distinguishable among the cladoceran taxa as indicated by similar relative frequencies of dispersal ($F_{4,20} = 2.01$, $p = 0.13$; Fig. 1.1). Additionally, trapped abundances of individual taxa were low (< 100 individuals, except chydorids = 393); thus, data were insufficient to apply mathematical models to individual taxa. As such I pooled all cladocerans for the remaining analyses. When summed across all buckets within a experimental run, these pooled count data were highly correlated with the density of cladocerans in the pond during that run (Appendix D); however, with only five points, the correlation was not significant (Pearson's rho = 0.84, $p = 0.16$). This high correlation is necessary for pond density to be used as a covariate to standardize individual runs in the dispersal modeling. Copepods also exhibited a similar relative dispersal rate (transformed mean ± 1SE: 0.098 ± 0.034). However, copepod dispersal did not positively correlate with pond density (Pearson's rho = -0.53, $p = 0.36$); thus, the copepod data could not be standardized among runs and applied to the dispersal modeling procedure. Cladoceran ephippia tended to disperse quite readily as their relative dispersal rate was high compared to all cladoceran adults (0.472 ± 0.125). However, total dispersal was much less than any active stage dispersing group and only occurred during four of five experimental runs. Finally, reproduction within traps was found to be non-significant (ANCOVA $F_{1,44} = 0.38$, $p = 0.54$; See Appendix A).

The second experiment suggested that animals were the primary vector for all zooplankton dispersal. Traps excluding large animals (> 3 cm wide) close to the pond (at 10 m) were colonized by 0 individuals, while open traps at 30 m were colonized by many

individuals (mean ± 1SE: 45.75 ± 31.90). Animals observed throughout both experiments (e.g., deer, raccoons and squirrels) also left visible signs of their presence in the traps (e.g., footprints, hair, large quantities of soil and gnaw marks). Together, these observations suggest that wind and rain were not important for dispersal at this site on this time scale.

1.4.2 Model Results

The distribution of cladoceran trap counts ranged from 0 to 132 individuals among the buckets. While most high counts were found at 10 or 30 m from the source, there was a great deal of clumping in the data, such that many traps at one distance might have zero individuals while a couple traps caught 30 or more individuals. This variability led to high deviance values for the fitted models (Table 1.1). Of the four empirical models, the 2Dt model fit better than the rest by greater than 10 ΔAIC units (Table 1.1). Relative to the other models, the 2Dt fit a shallow head and long thin tail with an intermediate curvature (Fig. 1.2). Despite model differences, each fit closely at measured distances to 60 m from the source, where there was less than a two fold difference among models (Fig. 1.2 inset). The shape of the dispersal kernel for the 2Dt model is shown in Fig. 1.3a over all pond densities. While individually fitting a curve to each replicate would produce five more closely fit models, here including cladoceran abundance as a regression variable likely provided the best overall estimate of dispersal, because it was a major contributor to the differences among replicates. Hence, the curve for each replicate is the same shape only with a different response magnitude (Fig. 1.3b-f).

The shape of each curve strongly influences the predicted area encompassing the dispersers. While all models predict that 95% of the dispersers travel less than 1 km, the extreme models (IP and NE) differ by almost a factor of 10 in radial dispersal distances (Table 1.2). The best fit model (2Dt) suggests that the experimental radius encompassed about 63% of the dispersers and that 95% of the dispersers traveled less than 789 m (Table 1.2).

Next, actual estimates of dispersers and percent dispersers were calculated. The 2Dt model projected 5.08% of individuals in the pond on any given day were dispersing. Among the four models, this estimate ranged from 2.24 to 11.8%, suggesting relative agreement among the models (Table 1.2). These figures suggest that thousands of

individuals dispersed on a daily basis. For example, during replicate 3 there were an estimated 1.18×10^7 individuals in the pond. These dispersal estimates suggest that 600,000 individuals left the pond daily, 30,000 individuals traveling beyond the 95% radial bound (but see Discussion).

1.5 DISCUSSION

1.5.1 Local Dispersal Characteristics

The magnitude and scale of local dispersal in this study suggest that adult zooplankton are transported over land with the potential to be redeposited in nearby ponds. Traps captured nearly 1200 zooplankton (excluding Bdelloid rotifers) over 45 days in 2.26 m^2 of traps. Although traps were only placed as far as 180 m from the pond (where 5 individuals were collected), local dispersal models suggest the geographic extent of dispersal was much greater. While the majority of dispersers only traveled short distances, three of the four models predicted significant numbers of dispersers could travel beyond the reach of the traps, with the best fit model suggesting 37% of dispersing propagules landed outside the capture radius.

These data contribute to the ongoing debate in the literature over whether dispersal among zooplankton communities is an inherently fast or slow process. Louette and De Meester (2004, 2005) noted fast colonization of their ponds (< 15 months) for a variety of cladoceran species and Cohen and Shurin (2003), studying a denser region of ponds, made similar conclusions. Jenkins (1995) and Jenkins and Buikema (1998), however, studying very isolated ponds, reached the opposite conclusion. It is highly likely that colonization in these studies resulted from a combination of adult and ephippial dispersal. My study suggests that the importance of adult relative to ephippial dispersal depends on the degree of isolation of ponds. Researchers have long assumed dispersal was facilitated by desiccation resistant diapausing eggs (Pennak 1989). This is a reasonable assumption, as adults may be incapable of surviving long distance travel due to drying. Yet, for the short distance dispersal studied here (and likely for Cohen and Shurin's (2003) study which just lasted a few weeks), adults were the primary contributor to colonization. This difference in the primary dispersal stage has large consequences for the rapidity of colonization. While adults can immediately grow and multiply, hatching

cues are necessary to begin the growth phase of resting eggs, cues which may take an entire season or longer to experience (Cáceres and Tessier 2003, Vandekerkhove et al. 2005). Ephippia were found to disperse at a high rate in this study; however, their absolute numbers were lower than the number of adult cladocerans dispersing (615 adults vs. 217 ephippia). This may reflect greater dispersal potential for ephippia and/or could be indicative of inaccurate water column density estimates (e.g., ephippia may sink to the pond sediment or may drift to pond edges, hence lowering pond density and increasing their likelihood of attaching to walking vectors). At the same time, adults may have greater opportunities to disperse, as they achieve much high densities and are present in the water column for longer periods of time than ephippia (which were only found in traps during replicates with high pond density).

An important contributor to the pattern described here was the fact that animals (> 3 cm) were the primary dispersal vector. Mammals, woodland birds and insects have a much smaller potential range over which to disperse propagules than wind, rain or long ranging animals such as waterfowl (e.g. Figuerola et al. 2005). Additionally, animals are more likely to engage in directed dispersal. While this suggests that the traps themselves may have attracted animals (see 1.5.2 Modeling Dispersal), it also means that animals are more likely than wind or rain to transport propagules directly from one source pond to another. If wind and rain were more prominent dispersal mechanisms, one would expect more dispersal at further distances than observed here (or more evenly distributed dispersal), as these processes tend to be more widespread than local animal movements. Thus, the abundance of animals here had an impact on both the magnitude and the physical distribution of the pattern observed.

Accordingly, in other areas, one might expect to see a different shape, magnitude and tail of the dispersal curve, depending on those animals present and the wind and rain conditions. For example, studies by Cáceres and Soluk (2002) and Cohen and Shurin (2003) suggested dispersal patterns were driven by local wind and rain conditions. Two major differences among our studies, the setting of the experiments and the specific animal vectors present, likely influenced these conclusions. Specifically, Cáceres and Soluk (2002) and Cohen and Shurin (2003) carried out exclusion studies at open field sites near permanent ponds, while this study was conducted in a forested site.

Additionally, the large animal vectors at Trelease Woods did not frequent (or were excluded from) the open field sites (Cohen and Shurin 2003, Fig. 5; C. Cáceres, pers. comm.). These location specific concerns equate with studies of plant dispersal where calculating dispersal curves at different times, during different wind conditions or with difference species affects the scale and magnitude of dispersal results (e.g. Turchin and Thoeny 1993; Skarpaas et al. 2004). Further work to assess the capability of mammals, birds and large insects to disperse zooplankton would help to address some of these location-specific differences.

1.5.2 Modeling Dispersal

The models discussed beg the question, how reasonable are the dispersal estimates presented in this study? Two points must be considered: the potential "attractiveness" of traps and how well the dispersal curve shape reflects reality. First, attractiveness reflects the area over which the trap collects, and one major assumption of these dispersal models is that traps do not attract propagules. However, as animals were the most likely dispersal vector at this site, a water filled bucket is certainly an attractive trap. To assess the potential influence of trap attractiveness on total dispersal, I estimated an upper bound of attractiveness by comparing catch among traps at 10 m. The distance between traps at 10 m ranged from 10.7 to 27.7 m. If the collecting area of the traps overlapped, I assumed the total catch among neighboring traps would be less at smaller distances than at larger distances. Hence, there would be a positive relationship between distance between traps and cumulative trap catch. No relationship would suggest that the trap collection areas did not overlap. I found no significant positive relationship for the overall experiment or any of the individual replicates (Appendix E). As such, one half the distance between the closest traps serves as a maximum upper bound of attractive area (a 5 m radius). Given that buckets each had a radius of 0.15 m, including maximum potential attractiveness would effectively increase the trap size 1000-fold. While attractiveness reduces the overall abundance of animals leaving the pond, it should not significantly influence the shape of the curve, suggesting the radial distance estimates would remain the same. Thus, a 1000 fold reduction would only reduce the maximum dispersal estimate to 600 individuals dispersing daily, 30 making it past the 95% radial limit.

A second potential issue is that fitting empirical curves to reflect accurately dispersal near and far from a source has proven a challenge in the past. Clark et al. (1999) tried to address this issue by proposing the 2Dt model, and in my case, this model provided a much better fit than the three alternatives (Table 1.1). Additionally, the fact that cusp of the curve is between 30 and 60 m is important, because it means there are two or three distances in the tail of the curve for which to fit the model. These points in the tail make extrapolating past the 180 m traps more reasonable. The 2Dt model, with its long tail, shallow curvature and thinner head, likely produces the best model fit because it is an intermediate alternative to the more standard dispersal kernels. Two other concerns for producing accurate dispersal models in this, or any, study are 1) over sampling the population at closer distances (i.e. removing individuals from the moving population that might have traveled a further distance) and 2) diluting the density over an increasingly broad radial area (as $area \propto r^2$) (Turchin 1998). Over-sampling is not likely a problem here, because the area covered by the traps is only a very small proportion of the total area. Attraction to traps might have accentuated this issue, but only if the attractive area was very large. However, density dilution always presents a challenge, and numerous ways have been suggested to cope with this phenomenon, such as increasing the area sampled with increasing distance or sampling sectors of constant angle over increasing distance (Bullock and Clarke 2000, Skarpaas et al. 2004). Unfortunately, those options were not available here, and as a result, there was great variability in density at the most distant traps. Thus, the small area sampled at these distances probably contributed to less precise density estimates at the tail of the distribution. Future attempts to measure zooplankton dispersal using this method would benefit from increased sampling area, a more direct estimate of the effective trapping area, additional dispersal distances and an optimized design strategy to improve the fit of the tail and to provide better data for estimates of longer distance spread (Skarpaas et al. 2005).

A third potential concern that would influence the model is zooplankton from other ponds being transported into the dispersal trap matrix. Indeed, three *Bosmina* were found in one trap during the study. As only one *Bosmina* was found in water column samples during the entire season (and in a different month than the trap collection), I assumed this to be a long distance dispersal event. Yet, the next nearest pond (400 m)

never contained *Bosmina*, suggesting this dispersal event occurred from ponds greater than 1 km away. This event lends credence to the possibility that adults can disperse for long distances. However, two pieces of evidence strongly suggest nearly all individuals trapped were transported from the source pond: the resulting shape of the dispersal curve reflects a source (i.e the majority of the trapped individuals were within 60 m of the pond), and the rest of the ~1200 individuals trapped were found in the source pond.

1.5.3 Consequences of Dispersal

Regardless of the extent of local dispersal, for long-established, stable communities, its influence on community composition may be limited. While some research suggests that high amounts of dispersal among extant local communities may be a structuring force (e.g., Cottenie et al. 2003), contributing to compositional similarity among neighboring ponds (i.e. a "mass effect"), the amount of dispersal necessary to structure local dynamics is probably quite high (perhaps up to 1.5% of individuals dispersing into a pond daily; Michels et al. (2001)). Shurin (2000), however, showed that despite high imposed dispersal, local communities were quite resistant to invading species. Local interactions or environmental conditions in extant communities likely led to long term species sorting where dispersal was not sufficiently high to directly influence composition. Evidence for high F_{ST} values among nearby ponds supports the proposition that dispersal has little influence on long-established communities (Boileau et al. 1992, Gomez et al. 2002).

Yet, reductions in the connectivity of aquatic habitats could still lead to a wide range of effects on metacommunity structure and function. Newly constructed ponds may be strongly structured by the species composition of established local communities (Louette and De Meester 2005). For highly fragmented or isolated new communities, fewer propagules will arrive in these systems allowing dispersal limitation and low genetic diversity to structure community assembly trajectories (De Meester et al. 2002). Boileau et al. (1992) suggested that the genetic consequences of strong priority effects could take thousands of years to overcome, even with moderate levels of dispersal. If dispersal levels remain low, reduced gene flow would bolster opportunities for local adaptation to specific environmental conditions and enhance potentially high genetic divergence values already noted in the literature (Boileau et al. 1992; Gomez et al. 2002).

Additionally, reduced movement could lead to selection against traits engendering dispersal (e.g., behavior modification, ephippial modification) . As habitats become more isolated, those individuals whose propagules leave the community are much more likely to be delivered to inviable terrain, effectively selecting those genotypes out of the population (Jenkins et al. 2003). This process might lead to selective tradeoffs developing, especially those favoring prolonged dormancy over dispersal as a more viable option for weathering temporally and spatially variable conditions (Venable and Lawlor 1980, McPeek and Kalisz 1998)

Our history of habitat destruction has likely reduced the role of adult dispersal in many areas. Central Illinois, for example, once had ~90% more wetland area than it currently boasts (Suloway and Hubbell 1994). This wetland destruction and corresponding reduction in the habitat for zooplankton dispersal vectors directly affected the amount of ongoing dispersal among communities, leading to the loss of eight or nine zooplankton species in Illinois (Jenkins et al. 2003). Yet, this research suggests animal-mediated adult dispersal may be an especially important component of the colonization process for areas with high pond densities. As efforts to restore lost or disturbed habitats intensify, ponds will become more connected and habitat for zooplankton dispersal vectors will increase, providing greater opportunities for adult dispersal and colonization. High local dispersal will provide opportunities for gene flow, reduce priority effects and mitigate potentially negative population effects such as inbreeding, while contributing to the long term composition of newly developed systems.

1.6 ACKNOWLEDGEMENTS

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1.8 TABLES

Table 1.1

Estimates of dispersal model parameters, residual deviance, Akaike Information Criterion (AIC) values and deltaAIC values for the four models (IP, MNE, NE, 2Dt) fit to the cladoceran data. The deltaAIC was calculated as the model AIC minus the minimum model AIC. The 2Dt model fit the data substantially better than the three alternative models. AIC weights for the alternative models were all less than 0.20%. All models were fit to n = 160 data points with 158 degrees of freedom.

Model	**A**	**B**	**Dev**	**AIC**	**ΔAIC**
IP	0.000023	1.05	1564.4	1772.5	12.41
MNE	0.0000071	64.94	1567.3	1775.3	15.21
NE	0.0000024	32.76	1599.2	1807.2	47.11
2Dt	0.0037	490.66	*	1760.1	---

*The ‘optim’ procedure does not provide a residual deviance estimate.

Table 1.2

Estimated dispersal distance and magnitude from Center Pond (Urbana, IL) in 2004 for four models (IP, MNE, NE, 2Dt) fit to the cladoceran data. r_{50}, r_{75}, and r_{95} are estimates of the radius enclosing that percent of all dispersers for a given model. The fitted percent estimates the fraction of individuals dispersing from Center pond on a daily basis.

Model	**r_{50}**	**r_{75}**	**r_{95}**	**Fitted %**
IP	439	715	943	11.8
MNE	59	114	234	2.78
NE	44	77	144	2.24
2Dt	101	310	789	5.08

1.9 FIGURES

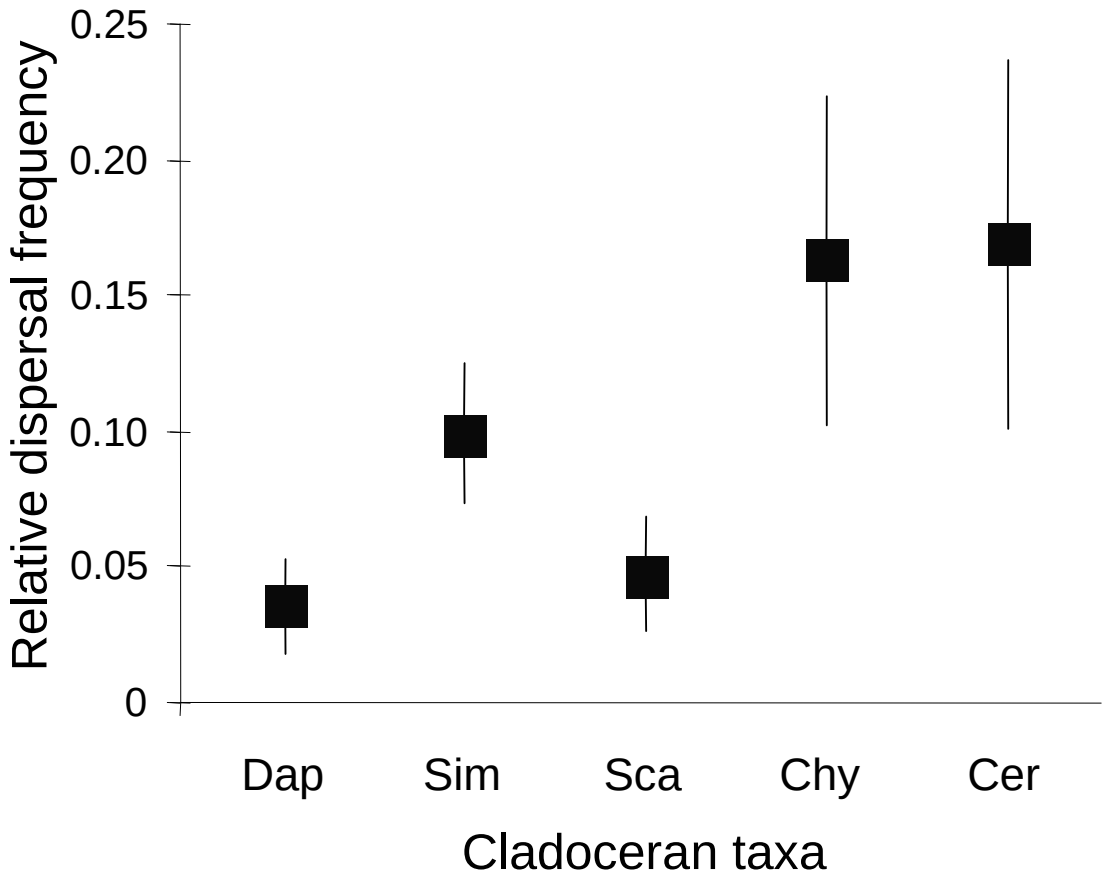

Figure 1.1

Relative dispersal frequency of cladoceran taxa from Center Pond (Urbana, IL) in 2004. The relative frequency (mean ± 1SE) is the arcsine-square root transformation of the total trap catch within a replicate divided by the average pond density within that replicate. There is no significant difference among taxa ($F_{4,20} = 2.01$, $p = 0.13$). Taxa: Dap = *Daphnia pulex*, Sim = *Simocephalus vetulus*, Sca = *Scapholeberis mucronata,* Chy = Chydorid spp., Cer = *Ceriodaphnia reticulata*

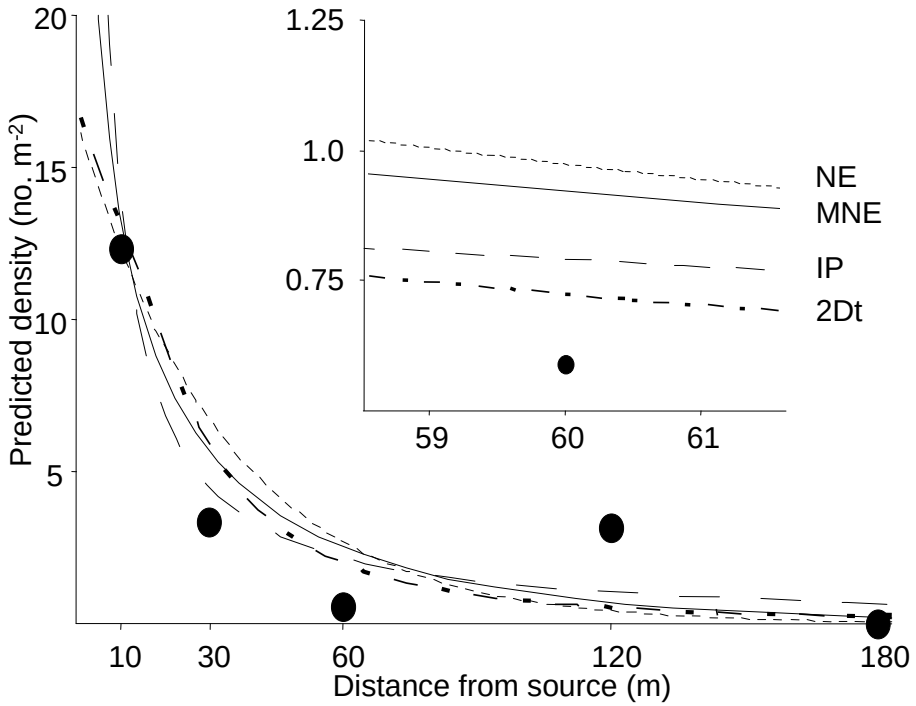

Figure 1.2

Observed (●) and predicted density of cladoceran individuals with increasing distance from pond source in four models: negative exponential (NE), Students' two-dimensional t (2Dt), mechanistic negative exponential (MNE) and inverse power (IP). The 2Dt model provided the best fit to the conglomerate data set. The fit shown is for the fourth experimental replicate. The inset figure shows the differences among the four curves in the tail of the kernel.

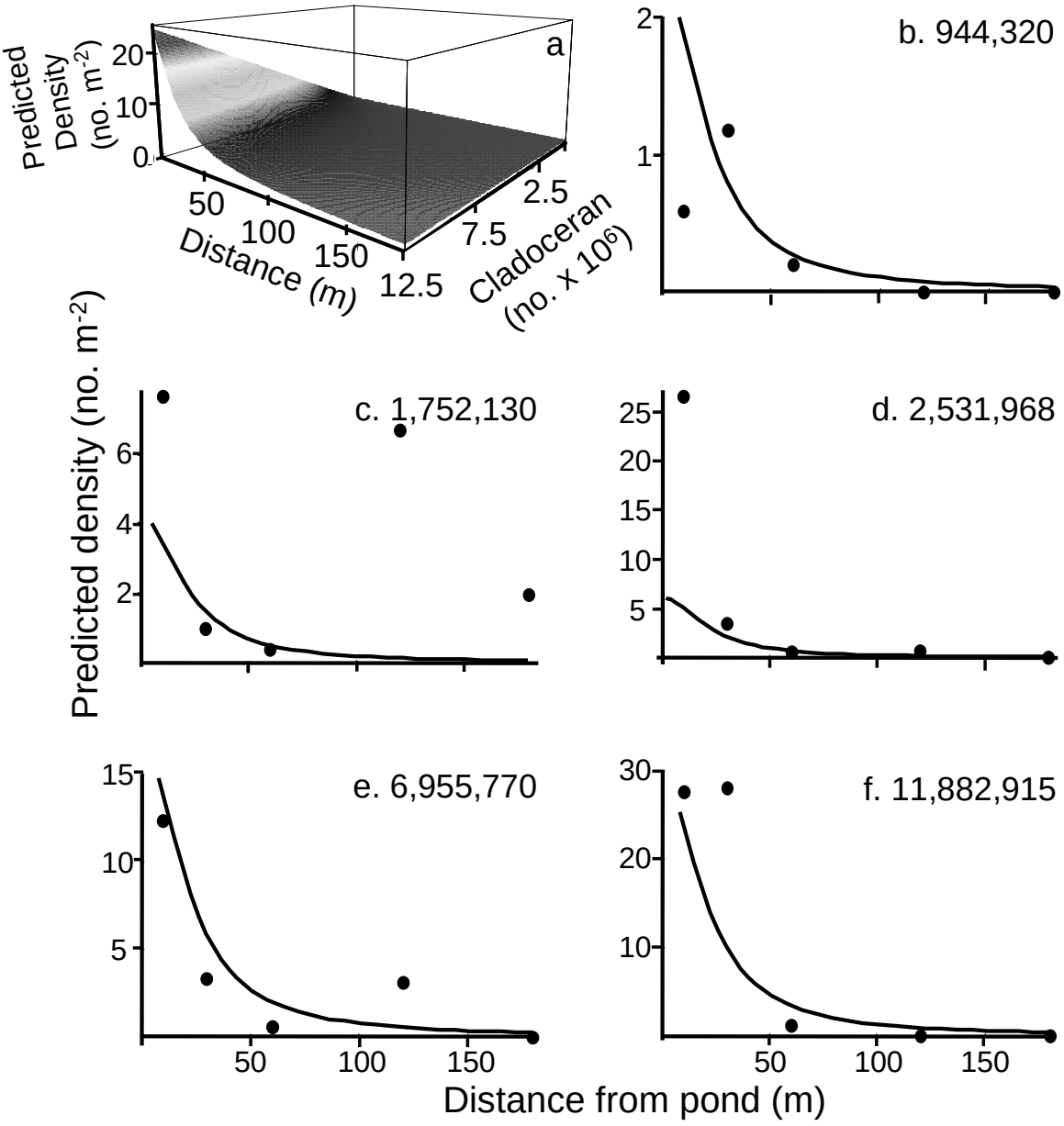

Figure 1.3

Fit of (a) the complete 2Dt dispersal kernel model over distance from the pond and cladoceran abundance (the total number of cladocerans in Center Pond on a given day) and (b-f) five replicates of the experiment at the following cladoceran abundances: b) 944,320, c) 1,752,130, d) 2,531,968, e) 6,955,770 and f) 11,882,915. Mean data for each distance during each experimental run are overlaid.

Chapter 2: Genetic and environmental factors influence survival and hatching of diapausing eggs

2.1 ABSTRACT

Many short-lived organisms persist despite temporal variation in reproductive success by incorporating prolonged dormancy into their life cycle. Although selection can shape optimal dormancy patterns, variation in the environment to which dormant eggs are exposed also influences observed hatching rates. Questions remain, however, regarding the relative importance of environmental and genetic influences on hatching rates in different habitats. Previous work on lake-dwelling *Daphnia* has demonstrated a lack of genetic differentiation among populations for this trait. I predicted that species from shallow ponds should experience greater access to hatching cues, and thus, more likely show genetic divergence or population by environment interactions for hatching rates. To test this prediction, I measured variation in prolonged dormancy and egg survival for *Daphnia* from 22 shallow, fishless ponds in the Midwestern USA. Although all eggs were incubated at a water depth of 0.75 m or less in their natal pond, hatching rates varied between 5 – 95% and survival rates ranged from 0 – 80%. There was no apparent relationship between hatching and environmental cues such as light, oxygen content or conductivity, although a negative relationship with depth was observed. Reciprocal transplant experiments quantified genetic and environmental influences on dormancy and survival, revealing strong population by host environment interactions. Thus, plasticity to environmental cues and genetic or maternal effects likely interact to determine hatching and survival rates in the field.

2.2 INTRODUCTION

For organisms living in seasonal or variable habitats, persistence depends on life history strategies that allow survival through unfavorable conditions. Dormancy is one common life history mechanism that allows propagules (e.g., seeds, diapausing eggs, statoblasts, quiescent adult stages) to "escape through time," surviving in an inactive state until favorable conditions return. Yet, breaking dormancy can be risky: individuals may receive emergence cues and return to an active phase in a habitat that cannot sustain them

long enough to reproduce. Given this risk, many taxa employ strategies whereby some fraction of offspring does not break dormancy at the first opportunity (e.g., desert annuals, insects, crustaceans: Philippi and Seger 1989, Ellner 1997). This "prolonged dormancy" spreads the risk across multiple generations leading to the buildup of propagule or seed banks (Templeton and Levin 1979, Hairston et al. 1995, Cáceres 1997). The optimal strategy is predicted to maximize the long-term geometric growth rate given the degree of unpredictability in the particular habitat (Cohen 1966). However, prolonged dormancy investment is one of multiple potentially coevolving life history strategies for individuals to maximize their long-term growth rate under variable conditions (e.g., dispersal among habitats and iteroparity – McPeek and Kalisz 1998). Thus, whether a propagule breaks dormancy depends on the interaction of several factors including coevolution with other traits, optimal life history tradeoffs (e.g., active and dormant survival probabilities) and exposure to the ecological conditions cuing emergence.

Early evolutionary models for dormancy assumed differences in survival in the dormant versus the active states selected for specific germination rates of propagules (Cohen 1966, 1967). Further theoretical work incorporated density dependence (Bulmer 1984, Ellner 1985), spatial and temporal variability (Levin et al. 1984, Kalisz et al. 1997) and tradeoffs with other important life history traits such as dispersal, adult longevity, and propagule size (Klinkhamer et al. 1987, Venable and Brown 1988, Rees 1994, Ellner et al. 1998, McPeek and Kalisz 1998). Particular theoretical attention has been paid to the relationship between optimal levels of prolonged dormancy and dispersal (Venable and Lawlor 1980, Levin et al. 1984, Klinkhamer et al. 1987, McPeek and Kalisz 1998). These models show a tradeoff between dispersal and dormancy: as the level of dispersal into alternate habitats increases, the optimal germination fraction also increases. All this work suggests prolonged dormancy significantly contributes to optimizing long-term fitness in variable habitats, but may exhibit complex interactions with other traits. As a result, predicting the expected level of dormancy for organisms in a particular habitat is challenging.

Many aquatic organisms have some form of dormancy (Hairston and Cáceres 1996), and the observation of large egg banks for many taxa suggests prolonged

dormancy also occurs (Destasio 1989, Hairston et al. 1995, Cáceres 1998, Brendonck and De Meester 2003). Eggs hatch in response to a variety of stimuli (e.g., predator chemicals, crowding, food quality, light, temperature – Gyllstrom and Hansson 2004) and there is evidence for differential responses to hatching stimuli at the population level (Schwartz and Hebert 1987, De Meester and De Jager 1993, Zarattini 2004). In some cases, models developed for seed banks seem to fit aquatic organisms well. For example, Simovich and Hathaway (1997) showed that dormant cysts of anostrocan species living in ephemeral pools fit the conditions for a diversified bet hedging strategy (Cohen 1966, Philippi and Seger 1989). However, for other habitats, prolonged dormancy may simply result from a lack of appropriate emergence cues (Brendonck 1996, Cáceres and Hairston 1998, Cáceres and Tessier 2003). As a result, the presence of egg banks may not represent an evolutionary strategy for persistence rather that the local environment simply prevents all eggs from immediately hatching. Thus, depending on the nature of the ambient environment, both evolutionary and ecological factors may influence the hatching fraction of aquatic organisms.

I examined natural variation in prolonged dormancy investment among *Daphnia* inhabiting small, fishless ponds in the Midwestern United States. *Daphnia* typically respond to light and temperature cues, and there is evidence that cue receptivity is partly under genetic control (Schwartz and Hebert 1987, Gyllstrom and Hansson 2004, Vandekerkhove et al. 2005). Previous work on lake-dwelling *Daphnia pulicaria*, however, found little evidence for genetic differentiation of hatching rates among populations in the field (Cáceres and Tessier 2003). They concluded that environmental conditions prevented access to the appropriate hatching stimuli, which limited hatching variation and effectively suppressed any observable genetic response or evidence of bet hedging. *Daphnia* in shallower habitats (e.g., ephemeral ponds), however, should have ready access to light and temperature hatching cues. Additionally, pond-dwelling *Daphnia* typically require annual reestablishment from the egg bank due to population crashes following pond drying and/or predation. Thus, while environmental cues likely continue to have strong effects on hatching rates, because those cues are more readily experienced in ponds, population-level differentiation in hatching may be more strongly expressed.

In this study, I address the following questions: 1) How variable are the hatching and dormant egg survival rates of *Daphnia* populations from shallow ponds? 2) To what extent is that variation controlled by environmental versus genetic factors? 3) Do specific limnological variables or evolutionary tradeoffs (e.g., with dispersal potential) influence the hatching rate? To address these questions, I surveyed the hatching fraction and dormant egg survival rate in 22 populations and used reciprocal transplant experiments in the field and in artificial common gardens to explore genetic and environmental influences on hatching and survival. Additionally, I used limnological and spatial variables to test potential predictors of hatching rates.

2.3 METHODS

2.3.1 Field Observations

Between 2005 and 2007, I visited a series of ephemeral or semipermanent fishless ponds in Illinois, Indiana and Michigan (Fig. 2.1) to assess environmental characteristics and to collect *Daphnia* diapausing eggs for hatching experiments. Beginning in late March 2005 (shortly after thawing), five ponds in central Illinois were visited every other week to monitor ephippial production of the resident *Daphnia* species. These populations were dominated by *Daphnia pulex* or *D. obtusa* in late spring, two morphologically similar species that are only easily distinguishable by molecular techniques (Hebert 1995). For each population, I used a 3 L pitcher and 70 μm sieve to collect 100+ L live samples from the water column during peak ephippial production. These samples were returned to the laboratory where ephippia were removed, dried and stored at 4°C for hatching experiments (see below). Subsets of these ephippia, or ephippia stored in ethanol samples on these dates, were dissected to calculate the initial percent of viable eggs (versus missing eggs) in ephippia from each population. Ephippia had a 90 – 98% viable egg filling rate among the populations.

In spring 2006, I sampled more than 60 ponds to identify those containing populations of *Daphnia*. As the Illinois populations were further south than the Michigan and Indiana populations, peak ephippia production occurred approximately one to two weeks earlier in the season. Ephippia were collected from the Illinois populations during 5 – 8 May 2006 and from the Michigan/Indiana populations during 14 – 25 May 2006

following the field and laboratory methodology outlined above. Of the initial 60 ponds, 22 contained populations of *Daphnia* from which I was subsequently able to collect sufficient numbers of diapausing eggs for hatching experiments (Fig. 2.1). Most ephippia contained viable eggs, ranging from 86 – 100% among the populations (Appendix F).

Limnological variables were also measured at the time of the egg hatching (early April 2007). Dissolved oxygen concentration, conductivit, temperature and pH were measured in the field. Chlorophyll content was estimated by filtering pond water through a 0.2 μm filter (Whatman GFF), extracting the chlorophyll in ethanol and measuring the absorbance using a Turner Designs 700 fluorometer (Welschmeyer 1994). Water samples were collected for total phosphorus content and analyzed in the lab following the molybdate-ascorbic acid extraction method (APHA 1980). Finally, I measured light conditions for each pond. Light was measured in the open, at and just below the pond surface and at the depth of the hatching trays (see below) using a LI-185B photometer (Li-Cor, Inc.). These light measurements were used to calculate a composite light variable – percent ambient light reaching the hatching tray.

2.3.2 Hatching Experiments

To determine the extent of prolonged dormancy for each population, I used the hatching methodology of Cáceres and Tessier (2003). Dried ephippia were placed into 6-well culture trays, which were covered with 200 μm mesh and sealed with a lid containing holes above each well (2.5 cm diameter). This design allowed water exchange but prevented egg loss. Trays contained 50-60 eggs (25-30 ephippia) and each represented a single experimental replicate. For the 2005 experiments, I collected sufficient quantities of ephippia to measure the in situ hatching fraction of five populations and to conduct a small reciprocal transplant experiment in the field. This design allowed me to test for differences in hatching among populations and to examine the roles of genetic and environmental controls on hatching in the field. In fall 2005, I placed eggs from each pond into two or three replicate hatching trays and secured those trays to their natal pond's sediment. Trays were placed approximately 0.3 m below the highest surface of the ponds (which were dry at the time), and allowed to overwinter in the field. Additionally, enough eggs were available to place trays of Center Pond eggs into Edge and Top ponds, Edge Pond eggs into Center and Top ponds, and Top Pond

eggs into Center Pond. Ponds filled with water and covered the emergence traps by the end of March 2006. Trays were removed in May 2006, and stored frozen to prevent any further egg development or decay until eggs could be checked. Once thawed, ephippia were dissected. Eggs were counted and scored as missing, present and viable, or present and inviable (Cáceres and Tessier 2003).

To measure hatching variation in the field in 2006, I followed a similar methodology. Eggs collected from the 22 ponds in spring 2006 were placed in hatching trays and returned to the field in November or December 2006, where they were allowed to overwinter. Trays were placed in the basin of each pond such that they would be covered with water in winter or early spring. During the April 2007 limnological sampling excursion, the depth of the trays below the water surface was measured as this distance could influence hatching cues the eggs experienced (i.e., light and temperature). Those trays were removed in late May 2007 and again stored frozen until they could be scored.

As a result of the high environmental variation observed in the 2005 field-based reciprocal transplant experiment, I set up dual common gardens in 2006 to better assess the extent of environmental versus genetic control on hatching variation among these populations. Common gardens were set up in southwest Michigan at the Kellogg Biological Station Pond Lab and in central Illinois at the Phillips Tract Natural Area during December 2006. Three replicate cattle tanks were established at each site and filled with ~0.5 m of well water (Fig. 2.2). One tray from each pond was secured to the bottom of each of the tanks. Each tank was covered with mesh or chicken wire to prevent animals from disturbing the experiment. Due to the locations of the field stations, important hatching cues such as light intensity and temperature differed between the common gardens during April 2007 (e.g., mean daily April temperature: Urbana 4 – 16° C, Kalamazoo 2 – 13° C – National Climate Data Center 2009). In late May 2007 the trays were removed and stored with those from the field and scored as above. From those populations that I had sufficient quantities of ephippia, I placed egg-filled trays in one or two of the common gardens. If insufficient quantities of eggs were available for both common gardens, trays were placed in the common garden closest to the source of the

eggs. In all, 14 populations were included in the Michigan common garden and 7 were included in the Illinois common garden. Five populations overlapped.

2.3.3 Data Analysis

From the hatching data, I calculated two vital rates that may be influenced by environmental factors and population genetic variation: the hatching fraction and the dormant egg survival rate. At the end of the incubation, each egg was classified into one of three categories: missing from its ephippium, viable or inviable. Hatching fraction is typically expressed as the number of eggs hatched relative to the total number of eggs. In an earlier study (Cáceres and Tessier 2003), hatching was calculated as eggs missing from ephippia divided by 2 times the number of ephippia recovered (two eggs per ephippium). However, in these experiments, less than 100% of the ephippia placed in the field contained two eggs. Thus, eggs that are missing from their ephippia either could have hatched or were missing to begin with. Additionally, less than 100% of the ephippia placed in the field were recovered. I corrected for these discrepancies when estimating the hatching fraction in the following way. First, I assumed that all eggs placed in the field were viable. This is a reasonable assumption based on examining freshly collected ephippia. Assuming that all eggs were viable meant that at the end of the incubation, all eggs were now hatched (missing), viable or inviable. However, I needed to account for the fact that a fraction of the eggs classified as "missing" at the end was missing to begin with. To do so I used the initial percentages of eggs missing from ephippia (calculated from freshly collected ephippia - $\%M_I$) to estimate how many of the "missing" eggs had in fact hatched. Thus, corrected totals of dormant eggs (viable) or eggs that died (inviable) were the final count divided by the percent of eggs that were not initially missing ($\frac{V_F}{\%V_I}$ or $\frac{I_F}{\%V_I}$), where $\%V_I$ equals 1-$\%M_I$. A corrected count of hatched eggs (H_E) was the total number of eggs missing after incubation (M_E) divided by the percent not initially missing ($\%V_I$) minus a correction for eggs missing to start with –

$H_E = \frac{M_E}{\%V_I} - \%M_I * T_E$, where T_E is the total number of eggs recovered from the field.

Thus, the corrected number of hatched, viable and inviable eggs added together equals the actual number of eggs recovered from the field. The corrected hatching fraction is

$\frac{H_E}{T_E}$, and both numbers (as opposed to the simple fraction) are required to run the statistical analysis. Survival of dormant eggs in the sediment is the corrected number of viable eggs recovered divided by the corrected sum of the viable and inviable eggs recovered. Because some populations had a very high hatching rate, the precision of this survival estimate is low due to a very small number of eggs to calculate the rate.

As hatching and survival rates of eggs from each population are proportions, I used generalized linear modeling with binomial errors and a logit link function (GLM) to correct for the non-normal variance structure (McCullagh and Nelder 1989). Analyses were run in the GENMOD procedure in SAS 9.2 (SAS Institute 2008) where response variables are the count of "successes" relative to the total number of trials (e.g., H_E:T_E). The procedure accepts positive non-integer values for counts. However, because the initial proportion of viable eggs placed in the trays ($\%M_I$) is an estimate, the corrected hatching count could be slightly negative if H_E was very low or $\%M_I$ was high. This happened for 2 of 124 hatching estimates in the 2007 experiments, and these counts were subsequently set to 0 (from -0.56 and -2.0). I corrected for overdispersion in these models using a quasi-likelihood function that estimated the scale parameter from the model deviance divided by its degrees of freedom (Littell et al. 2002). The significance of each variable was tested with a Type 3 analysis, which uses quasi-log likelihood contrasts to determine whether the addition of the variable significantly reduces the deviance of the model. F-tests were used due to the overdispersion correction.

For both the 2005 and 2006 datasets, I had many more estimates of hatching and survival from ponds in the field than in the common garden experiments. As such, for each dataset I used all of the data for populations incubated in their own pond to test whether there were differences in survival and hatching among populations in the field. This simple model treated "pond" as a fixed variable, and combined the environmental and genetic components of variance. I also used the regional (2006) dataset to test the effect of a number of environmental variables on hatching and survival rates. To control for pseudoreplication, I created a mean hatching variable for each pond by summing the hatched and total number of eggs across the within pond replicates. This provided mean count data for the binomial regression analysis with 22 pond replicates. I tested variables

known to influence hatching of aquatic crustacean diapausing eggs. For hatching, I tested the following four hypotheses: 1) increased conductivity reduces hatching (Spencer and Blaustein 2001), 2) increased light reaching the eggs increases hatching (Schwartz and Hebert 1987), 3) increased tray depth (a composite variable of light and temperature) decreases hatching, and 4) pH or dissolved oxygen concentration influences hatching (De Meester 1993, Brown 2008). I did not have the appropriate data to test for a direct effect of temperature. For survival, I tested whether pH or oxygen concentration influenced egg survival rates (De Meester 1993). Given the range of the study, I also tested for a longitudinal effect on hatching and survival.

I used a two-way GLM analysis to test the hypothesis that genetic by environmental interactions influenced survival and hatching of eggs. Using the reciprocally transplanted trays from Center, Edge and Top ponds in 2005, I examined the interactions between environment and population source for eggs incubated in the field. Population source and host pond were crossed and treated as fixed variables in the analysis. The 2006 common garden experiments were analyzed in a similar way. I used data from the five populations incubated in both Michigan and Illinois with common garden (CG) and natal pond (P) as variables.

Finally, I tested the evolutionary hypothesis that dormancy and dispersal potential were negatively correlated (Venable and Lawlor 1980, Levin et al. 1984). I used the hatching data from the Michigan common garden, because I had 14 hatching estimates (relative to 7 in the Illinois common garden) and the common garden provided more control of environmental variance than the field data. To measure the potential for dispersal among ponds, I calculated the total number of neighboring habitats within 1 km of each focal pond. Previous work has suggested this distance is a good approximation of a local zooplankton dispersal kernel (Allen 2007). Neighboring habitat frequency was counted using both aerial photos and the National Wetlands Inventory (US Fish and Wildlife Service 2006) in ArcGIS Desktop 8.1 (ESRI) and visual inspection onsite. I used a GLM to test for a direct effect of neighbor frequency on the hatching fraction.

2.4 RESULTS

Hatching rates in the field varied substantially among the ponds for both the 2005 and 2006 cohorts, but all populations exhibited some amount of prolonged dormancy (Appendix G, Fig. 2.3a). In the smaller 2005 sample, hatching fraction ranged from 50 – 92% ($F_{4, 9} = 13.6$, $p = 0.0009$), while in 2006, between 5 and 90% of the viable eggs hatched across the region ($F_{21, 39} = 10.70$, $p < 0.0001$; Fig. 2.3a). For the 2006 regional sample, there was no longitudinal effect on the mean hatching rate ($F_{1, 20} = 0.04$, $p = 0.85$). Additionally, there was no direct effect of conductivity ($F_{1, 20} = 0.08$, $p = 0.77$), pH ($F_{1, 20} = 0.32$, $p = 0.58$), percent ambient light reaching the trays ($F_{1, 20} = 2.05$, $p = 0.17$) or dissolved oxygen ($F_{1, 20} = 0.12$, $p = 0.73$) on mean hatching fraction. However, trays further beneath the water surface had lower hatching rates ($F_{1, 29} = 4.41$, $p = 0.05$).

Dormant egg survival rates were variable in the larger 2006 sample ranging from 0 to 72% (Fig. 2.3b; $F_{21, 39} = 2.34$, $p = 0.0097$), but much higher and not different from one another in the five ponds surveyed in 2005 ($F_{4, 9} = 0.65$, $p = 0.64$; Appendix G). However, estimates from populations with hatching fractions greater than ~75% should be treated with caution, as hatching reduced the number of eggs contributing to estimates of viability past the first year to 10 or less. Neither pH nor oxygen concentration significantly reduced the likelihood of egg survival in the regional survey using the mean dataset, though survival trended lower under low oxygen concentrations (pH: $F_{1, 20} = 0.56$, $p = 0.46$; oxygen: $F_{1, 20} = 3.64$, $p = 0.07$).

The source of the eggs and the local environmental conditions influenced hatching and survival in the field reciprocal transplant experiment (Fig. 2.4). Center and Edge ponds had similar hatching fractions in the three environments, and the lower mean hatching rate of Top Pond eggs drove the main effects (Fig. 2.4a). Both population ($F_{2, 13} = 13.10$, $p = 0.0008$) and host environment ($F_{2, 13} = 10.32$, $p = 0.0021$) significantly influenced hatching, but the interaction effect was not significant ($F_{3, 13} = 1.54$, $p = 0.25$). Survival rates were high in the field and similar among each of these populations. Thus, there was no effect of population ($F_{2, 13} = 0.64$, $p = 0.55$), host environment ($F_{2, 13} = 1.50$, $p = 0.26$) or their interaction ($F_{3, 13} = 0.44$, $p = 0.73$) on survival.

In the Michigan-Illinois common garden experiment, I found a significant interaction of population and environment for both hatching and survival (Table 2.1). The

mean survival and hatching rates were higher in the Illinois common garden (Fig. 2.5), but one population (PC2) had the opposite trend. This population also had the lowest hatching rate in the field (Fig. 2.3a), with a high mortality of eggs (72% died rather than hatched or remained dormant). These high death rates were also observed in both common gardens (IL: 70%, MI: 67%). Higher death rates were generally observed in the Michigan relative to the Illinois common garden ($F_{1, 19} = 39.41$, $p < 0.0001$). This may be attributable to a high mean pH observed in the Michigan common gardens at the conclusion of the experiment (pH = 9.3 ± 0.3 SD). The Campground population had much lower egg death rates in both common gardens, which contributed to the higher hatching and survival rates observed. Standard error rates were considerably reduced relative to the field study. These results suggest strong effects of both genetic background and host environment on the hatching and survival of the eggs.

Finally, I found a strong relationship between the hatching fraction of populations in the Michigan common garden and the number of neighboring habitats (potential for successful dispersal) in the field (Fig. 2.6; $F_{1, 12} = 8.91$, $p = 0.0114$). However, the relationship was in the opposite direction from that predicted by theory (Venable and Lawlor 1980, Levin et al 1984, Klinkhamer 1987, McPeek and Kalisz 1998). This relationship remained marginally significant when the pond with the most neighbors was excluded from the analysis ($p = 0.07$).

2.5 DISCUSSION

The ephemeral populations in this study exhibited substantial hatching variability with fractions ranging from almost zero to near 100% in a single season. Additionally, viable eggs were recovered from each population after the hatching season, suggesting eggs remain in the sediments in a prolonged dormant state. These experiments also suggest a large proportion of variability could be attributed to environmental variation among sites. First, incubation depth – a composite variable partially accounting for temperature and light differences among incubation sites – was negatively related to hatching fraction, suggesting eggs incubated further below the pond surface experienced reduced exposure to hatching cues, a pattern also observed in lakes (Cáceres and Tessier 2003). Second, there were large differences in hatching rates between the two common

gardens for the 2006 cohort, with generally higher hatching and survival rates in the Illinois common garden. However, unlike previous work (Cáceres and Tessier 2003), the 2005 and 2006 data also provide evidence that genetic or maternal effects contribute to hatching variation. Populations incubated in common gardens had distinctly different mean hatching and survival rates from one another in many cases. Additionally, there was evidence for genetic by environmental (GxE) effects in the 2006 experiment.

The reciprocal transplant experiments provide evidence that environmental factors act on hatching and survival. Previous work has shown temperature and incident light cues are important for achieving maximum hatching rates (Schwartz and Hebert 1987, Pfrender and Deng 1998, Vandekerkhove et al. 2005). For shallow ponds, a variety of factors can influence exposure to these cues, including canopy cover, pond size, egg depth and turbidity. Among pond variation in measured characteristics was substantial in this study. Yet, only tray depth explained any variation in hatching or survival among field populations (a direct metric of temperature was not available). There are a number of possibilities for this lack of a predictable response to incident light. First, while hatching experiments carried out in a laboratory can readily control the exact cues an egg experiences (e.g., Cáceres and Schwalbach 2001), the environmental measurements at the hatching trays may be too course to capture the cues experienced by the eggs. Individual ephippia may experience different microenvironmental conditions, contributing to intra-population variation (e.g., the wells of some trays may fill with sediment). Second, photoperiod, as opposed to light intensity, may be a more important cue for hatching, and photoperiods were similar among ponds in the region. Alternatively, mean hatching temperature or temperature variation, or their interaction with light, may have been stronger environmental cues (Pfrender and Deng 1998, Arnott and Yan 2002, Vandekerkhove et al. 2005) as is the case for many anostrocan species in similar environments (Brendonck 1996). Finally, genetic and GxE control of hatching behavior may confound the field observations. If underlying genetic differentiation sets different mean hatching fractions for each population, these values must be accounted for in any statistical analysis. Genetic by environmental interactions would further complicate the observed response in the field by altering the direction or magnitude of the environmental response.

Estimates of dormant egg survival also varied widely among populations in both the field and in the common gardens. Survival was generally higher in the field than in the common gardens, suggesting conditions were harsher in the common gardens. The high pH observed in Michigan may have led to reduced survival rates. The measured pH of 9.3 greatly exceeded the pH observed in most ponds (6.8 – 9.6), and high pH conditions are known to reduce viability of parthenogenetic eggs (Vijverberg et al. 1996). A variety of factors could cause variation in egg survival among populations. For example, pond depth, suspended or dissolved organic matter, and canopy cover may affect exposure to UV light, which has been shown to alter survival rates and induce melanization in *Daphnia* clones (Hessen et al. 1999). High dissolved oxygen content has also been shown to increase hatching rate but decrease survival (De Meester 1993, Cáceres and Tessier 2003). Additionally, many ponds in this study were small and/or isolated (e.g., PC2 is < 3 m^2). Such populations may exhibit varying levels of inbreeding, which also affects egg emergence and survival rates (De Meester 1993, Pfrender and Deng 1998).

Perhaps most interesting is the observation of partial genetic control of hatching variation among the populations. This result could be due to selection for an optimal germination strategy or a bet hedging response (Cohen 1966, Philippi and Seger 1989), adaptive phenotypic plasticity or a selective response to particular environmental cues (e.g., "predictive germination" – Cohen 1967), or inherent differences in initial egg quality among the populations (i.e., maternal effects – De Meester and De Jager 1993). The evolution of optimal germination strategies has been documented for a number of ephemeral systems (e.g., desert annuals, anostrocans), following a predicted relationship between the frequency of failed active stage reproduction ("catastrophes") and dormancy emergence (Philippi 1993, Simovich and Hathaway 1997, Clauss and Venable 2000). For ephemeral pond species, variation in hydroperiod or the onset of pond drying is a strong selection pressure influencing the evolution of hatching rates (Belk 1977, Brendonck 1996, Simovich and Hathaway 1997). However, of the ponds in this study, few typically dry prior to the ephippial production date (early to mid-May), suggesting hydroperiod is an unlikely cause of catastrophes for most populations (S. Smith, pers. comm.; M. Allen, unpublished data). If catastrophe frequency does influence the evolution of emergence in

these populations, it is more likely that other factors (such as temporal variability in the timing of predator dominance - sensu Hairston and Dillon 1990) act as selective forces, but such data are not available. Alternatively, germination strategies may coevolve with other traits (e.g., dispersal potential and propagule size - Venable and Lawlor 1980, Rees 1994, Ellner 1997). When I investigated such a relationship, hatching rates in the common garden were positively correlated with dispersal potential, the opposite direction predicted by theory. This suggests 1) at the low levels of dispersal exhibited among these ponds, the predicted relationship no longer holds, 2) dispersal potential is correlated with another trait that is related to dormancy, or 3) the GxE interaction makes the hatching values inappropriate for the test. Given the observation of GxE interactions in the two common gardens and the high variability of hatching in the field, it is likely that any effects of spatial dispersal are complex and interact with other selected traits and stochastic environmental variation.

The interaction of genetic and environmental factors provides support for a role phenotypic plasticity and genetic determinism on hatching variation. An interaction between adaptation and differential plasticity to hatching cues has been clearly demonstrated in desert annuals along a precipitation gradient. Clauss and Venable (2000) showed populations responded differently to water inundation in a common garden, whereby those experiencing less precipitation in the field were more sensitive to such events. Those populations from wetter sites had lower germination rates in the common garden, but had inherently higher germination success in the field due to greater overall precipitation. Such genetic by environmental variation in the germination response allows populations to grow when conditions are most favorable for survival, a form of predictive germination (Cohen 1967, Pake and Venable 1996, Clauss and Venable 2000). Pond dwellers may exhibit similar patterns. For anostrocans, there is considerable variation among species and habitats for response to hatching cues (Belk 1977). Some have attributed this variation to Cohen's (1966) model of differential probabilities of reproductive success during pond filling events (Brendonck 1996, Simovich and Hathaway 1997), but there is ample evidence that hatching rates vary in response to a range of cues (e.g., Brown and Carpelan 1971, Al-Tikrity and Grainger 1990, Brendonck

1996, Zarattini 2004). The observation of differential hatching responses in this common garden experiment fits such a scenario.

The genetic effect on hatching differences among populations may also be driven by variation in the environment under which the diapausing eggs were formed. Total phosphorus values varied by 10-fold and total chlorophyll values varied by greater than 100-fold across these ponds during the early spring. The variation in food availability is known to influence the mother's nutritional state, which can influence the fitness of her offspring (Brett 1993). Additionally, maternal effects can influence the hatching rate of her diapausing eggs (De Meester and De Jager 1993). Such maternal effects could be manifested as genetic or GxE effects as observed here, as the experimental design did not permit controlling for such effects.

I have shown substantial variation in hatching and survival rates of diapausing eggs, both incubated in the field and in common gardens. That there was no overwhelming signal for a particular environmental hatching cue suggests a variety of ecological and genetic factors interact to determine actual hatching rates. While adaptive bet hedging may contribute to such variation, local adaptation to hatching cues, adaptive plasticity (predictive germination), maternal effects and variable access to hatching cues provide alternate evolutionary and ecological explanations that can explain the observed patterns. Future work controlling for maternal effects, genetic background and cue exposure will help elucidate the relative importance and interactions among these competing hypotheses for regional hatching variation.

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2.8 TABLES

Table 2.1

Reciprocal common garden experiment. Tests for the interactive effects of genetic (Pond – P) and environmental (Common Garden – CG) variance on hatching and survival in two common gardens.

Hatching Fraction

Effect	**df**	**error df**	**F**	**p**
P	4	19	14.84	<0.0001
CG	1	19	33.68	<0.0001
P*CG	4	19	5.96	0.0028

Survival Rate

Effect	**df**	**error df**	**F**	**p**
pond	4	19	7.66	0.0008
CG	1	19	0.00	1.0000
P*CG	4	19	3.93	0.0173

2.9 FIGURES

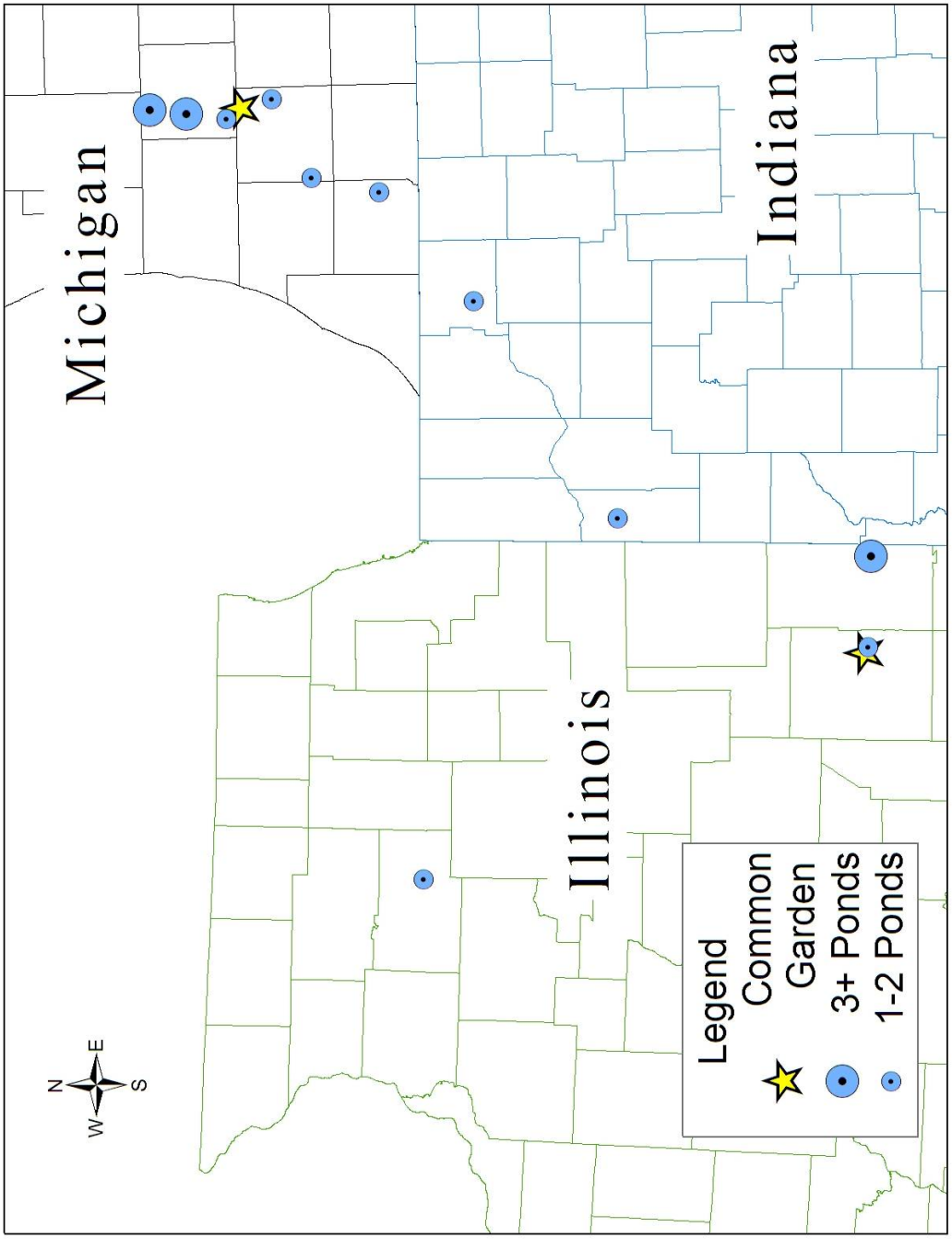

Figure 2.1
Hatching fraction experiment field sites. The Philips Tract (Illinois) and the Kellogg Biological Station (Michigan) common gardens

Figure 2.2

Plates of common gardens at the a) Kellogg Biological Station and b) the Philips Tract Natural Area. Hatching trays were secured to the bottom of cattle tanks with plastic fencing and weights. Photo (a) courtesy of Sigrid Smith.

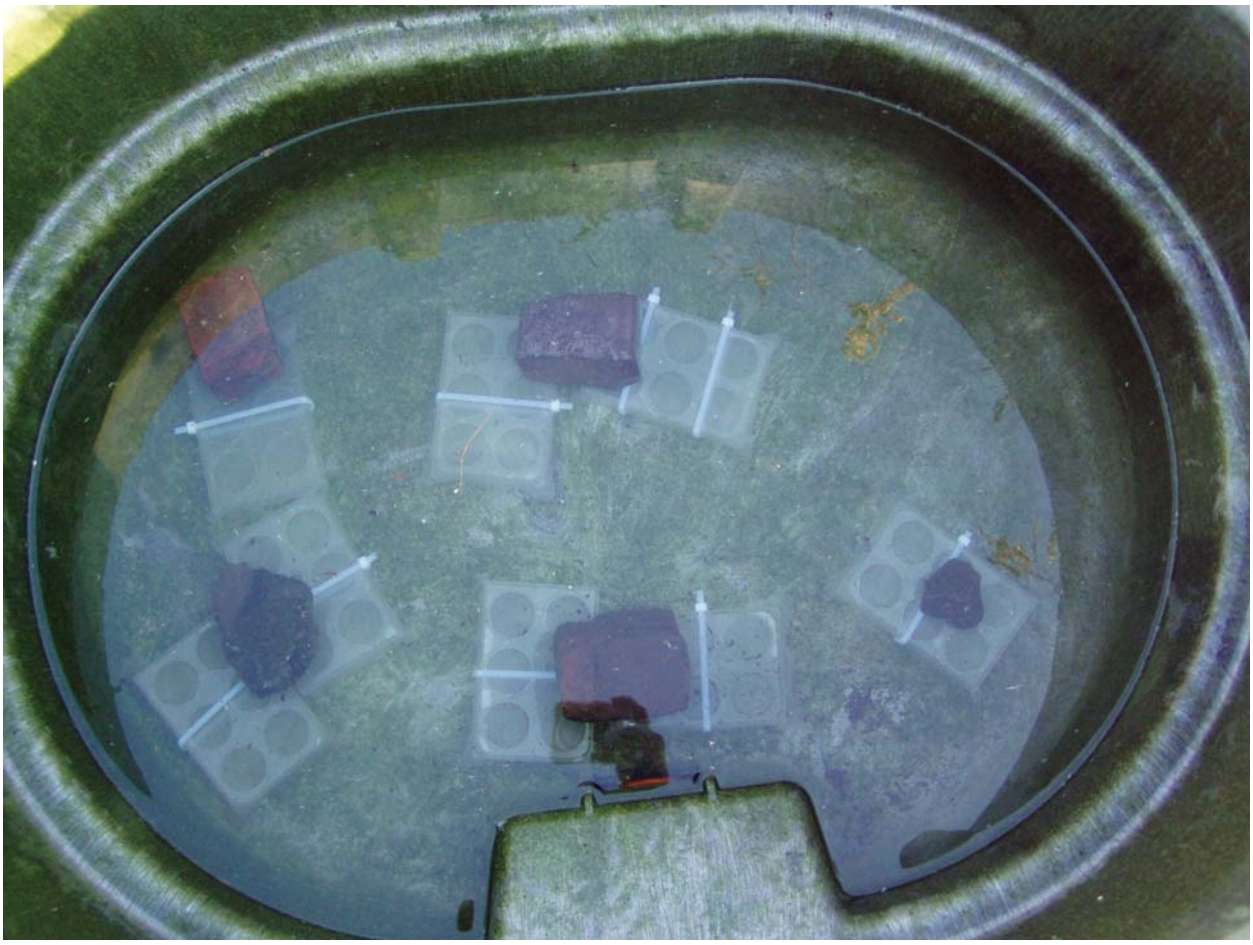

Figure 2.2 (cont.)

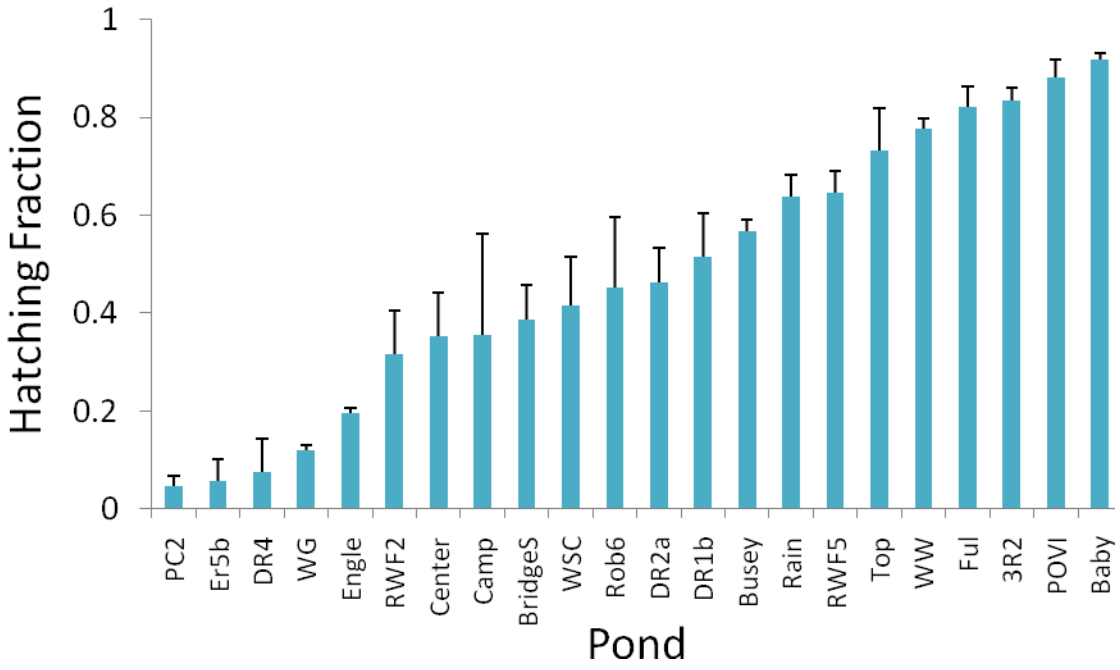

Figure 2.3

Mean (± SE) a) hatching fraction and b) dormant egg survival rate of field collected eggs incubated in their own pond during 2006-2007. Both figures are sorted from lowest to highest hatching fraction.

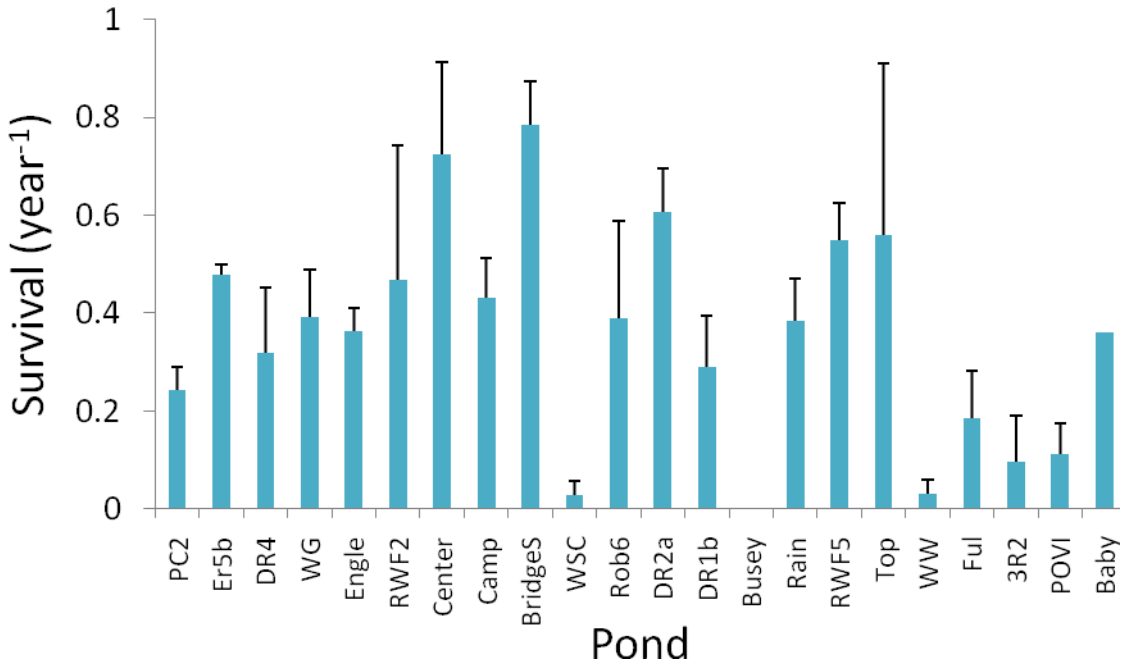

Figure 2.3 (cont.)

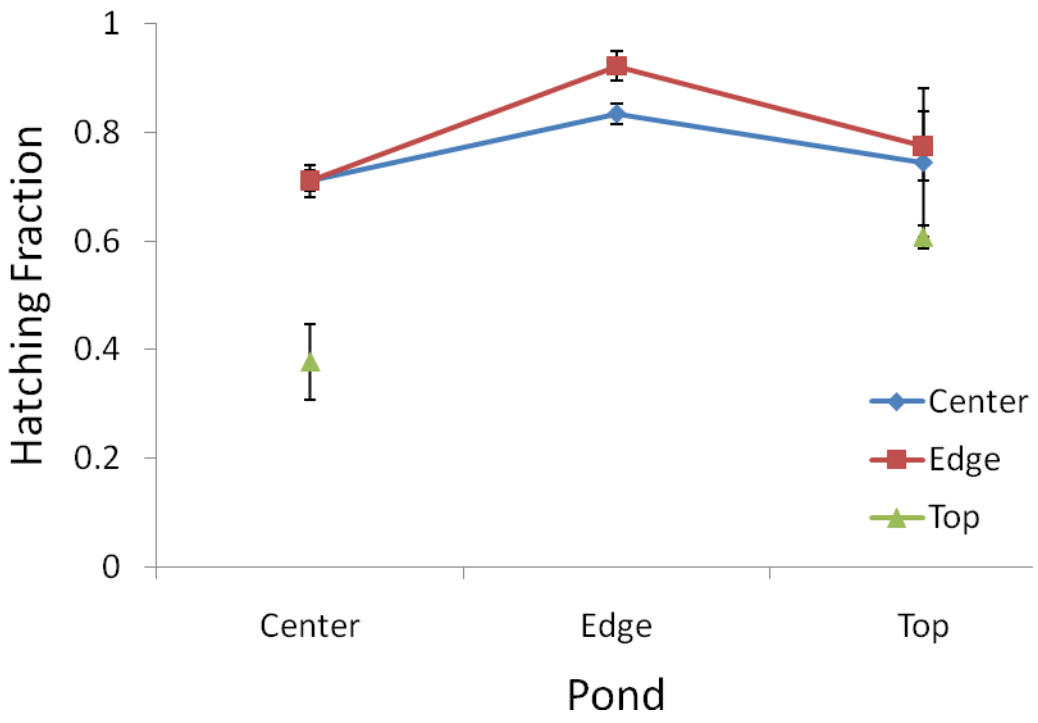

Figure 2.4

Reciprocal transplant of field collected eggs among three ponds during 2005-2006. Mean (± SE) of the a) hatching fraction and b) dormant egg survival probability.

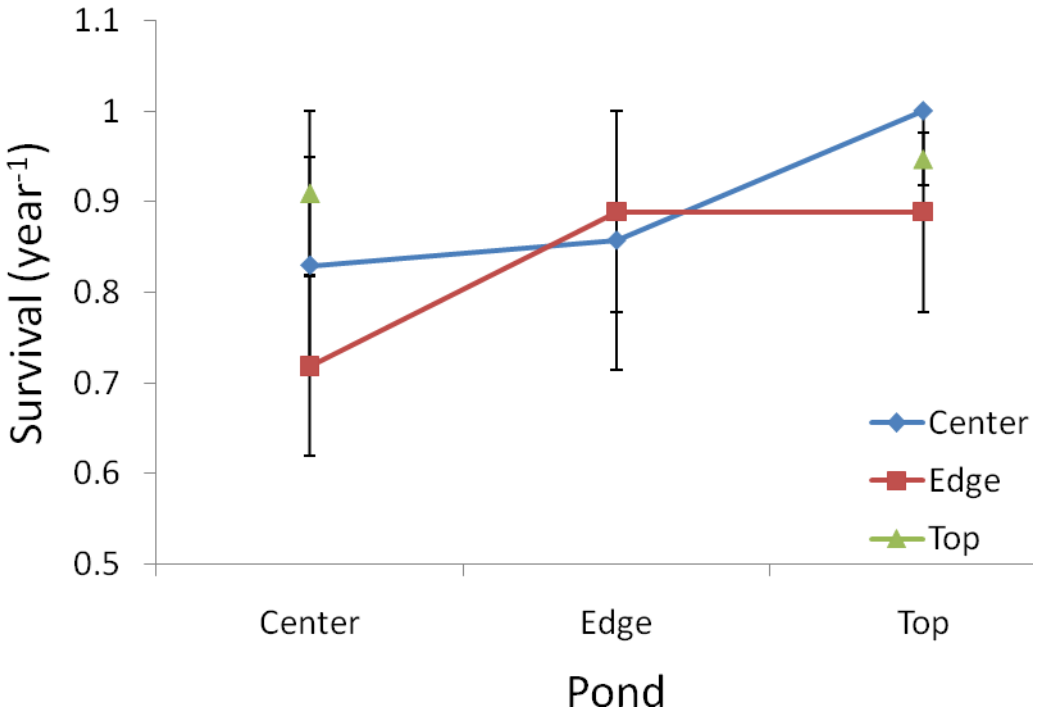

Figure 2.4 (cont.)

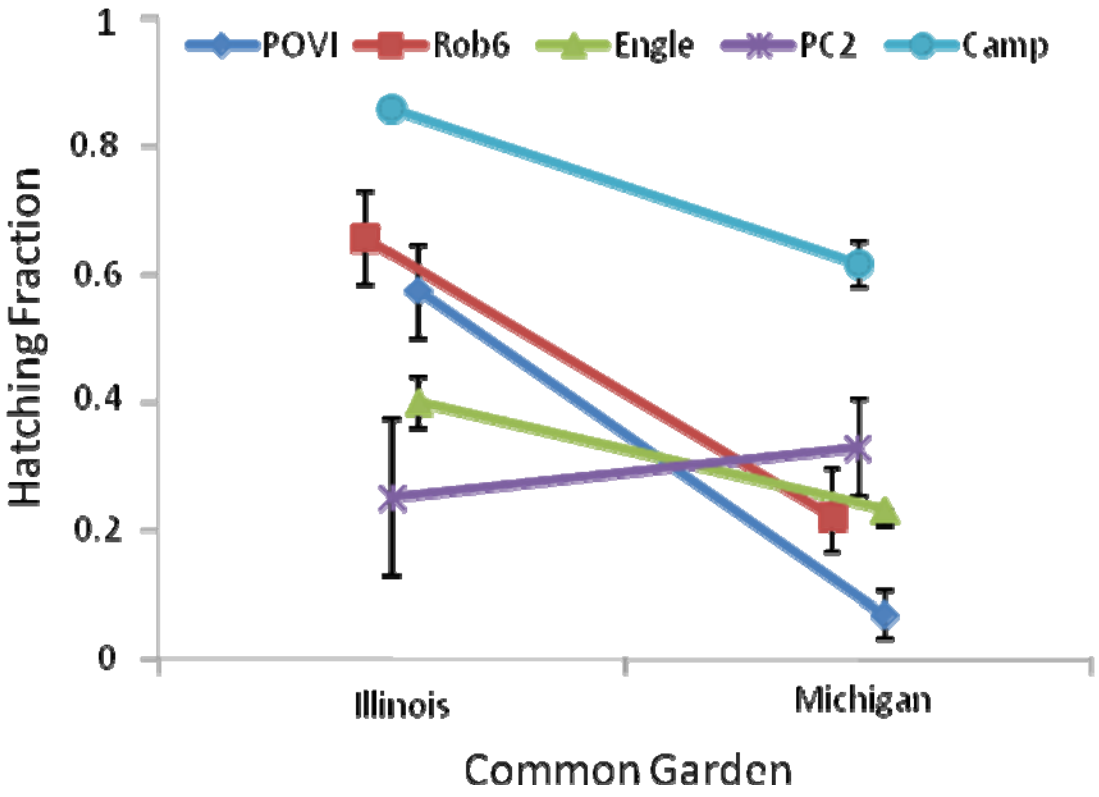

Figure 2.5

Reciprocal transplant of field collected eggs in two common gardens during 2006-2007. Mean (± SE) of the a) hatching fraction and b) survival probability of dormant eggs.

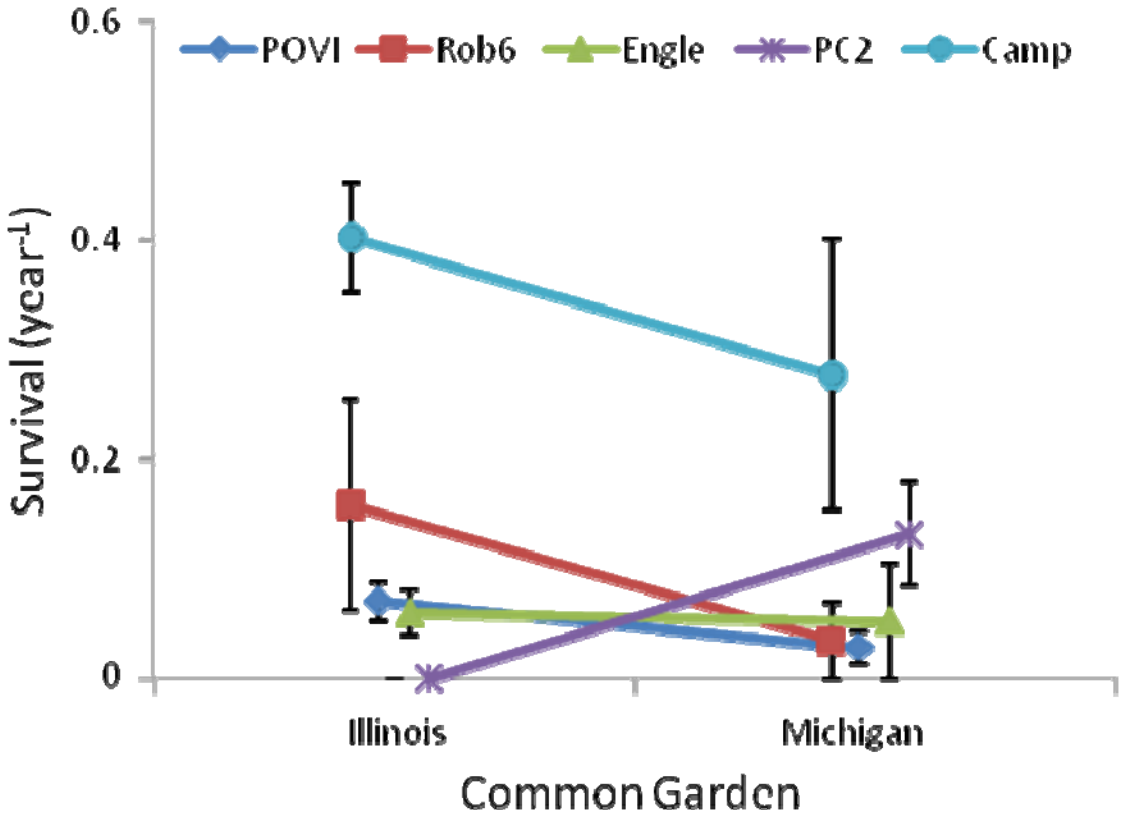

Figure 2.5 (cont.)

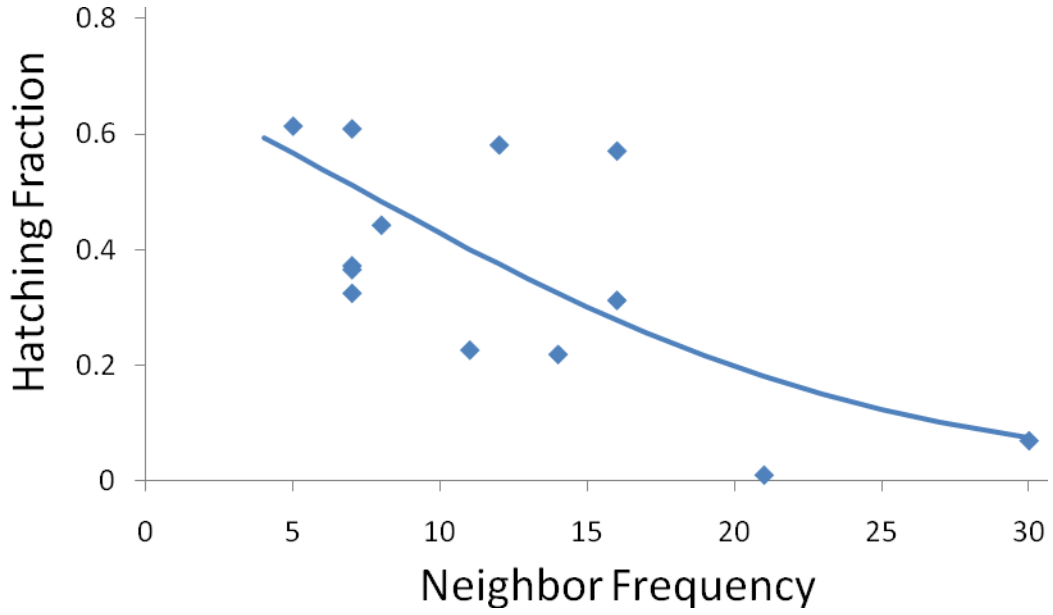

Figure 2.6

Relationship between the number of neighbors within one kilometer of a pond and the hatching fraction exhibited by that population in the Michigan common garden. The linear prediction was fit using a generalized linear model with binomial errors and a logit link function.

Chapter 3: Does resource monopolization explain genetic differentiation in *Daphnia* populations?

3.1 ABSTRACT

Substantial genetic differentiation is frequently observed among populations of cyclically parthenogenetic zooplankton, despite the potential for dispersal. Both the persistent founder effects hypothesis (Kolb et al.) and the Monopolization hypothesis have been proposed to explain this population structure. The PFE predicts this differentiation is the result of long lasting priority effects that have yet to be overcome by dispersal, whereas the Monopolization hypothesis assumes local adaption is also necessary to develop and maintain population divergence. Here, we ask which hypothesis best explains the patterns we observed in four populations of *Daphnia pulex* from central Illinois. We found moderate to high genetic differentiation among the populations (mean $\theta = 0.22$). However, genetic diversity within populations was also high (mean $H_E = 0.62$), many alleles were shared among populations, and the frequency of private alleles was low. These results suggest founder effects continue to influence population structure, but gene flow is occurring across the region. We then asked if local adaptation to resources contributed to the observed genetic divergence. Although both resource quantity (as measured by chlorophyll a) and quality (as measured by a bioassay) differed among the ponds, we found no evidence for local adaptation to resources. The mean juvenile growth rate was not highest on natal resources and none of the clones from any of the populations exhibited a genetically controlled response to a resource gradient. Instead, genotypes showed a plastic linear response to resource richness, implying that variation in growth rate does not contribute to population differentiation. Our results suggest gene flow is slowly eroding persistent founder effects, and at least in this system, resource monopolization does not explain observed patterns of population differentiation.

3.2 INTRODUCTION

Evolutionary biologists have long pondered the reasons underlying the divergence of allopatric populations. In the time since Fisher (1958) and Wright (1932) debated the relative influence of natural selection and stochasticity in driving evolutionary dynamics, we have come to understand that natural selection, mutation, migration and genetic drift

interact with ecology to contribute to population structure. Dispersal is particularly important as it directly influences the effects of drift and selection. For example, high dispersal rates can break down both stochastic and adaptive diversification among populations, and in extreme cases, lead to the homogenization of regional gene pools (e.g., Fuller et al. 1996, Roslin 2001, Gilbert-Horvath et al. 2006). Under lower dispersal regimes, selection and drift are less quickly counteracted, resulting in increased population genetic differentiation (Ehrlich and Raven 1969, Slatkin 1985). Understanding the relative importance of and interactions between these processes for organisms of diverse life histories is key to rigorously testing theories for evolutionary and ecological diversification in nature.

Zooplankton, especially cladocera in the genus *Daphnia,* are particularly attractive for empirical studies of the mechanisms underlying population differentiation. Previous work suggests zooplankton are capable of moving among ponds or lakes and rapidly colonizing habitats (Cáceres and Soluk 2002, Havel et al. 2002, Cohen and Shurin 2003, Louette and De Meester 2005, Johnson et al. 2008). This dispersal capacity suggests the potential for high gene flow, which should act to homogenize populations. Yet, *Daphnia* often exhibit strong population genetic differentiation, even over small geographic scales (Boileau and Hebert 1988, Spitze 1993, Vanoverbeke and De Meester 1997, Lynch et al. 1999, Morgan et al. 2001, Gomez et al. 2002, Haag et al. 2006). This observation of remarkable genetic subdivision in the face of potentially high ongoing dispersal has led to two complementary hypotheses to explain the mechanisms underlying differentiation: the persistent founder effects hypothesis (Kolb et al.) (Boileau et al. 1992) and the Monopolization hypothesis (De Meester et al. 2002). These hypotheses provide theoretical explanations for observations of population genetic differentiation, but distinguishing the mechanisms underlying them is challenging in practice.

Boileau et al. (1992) developed the persistent founder effects hypothesis based on observations of population genetic structure in copepods. The population genetic divergences they observed among nearby populations were indicative of low dispersal rates (Boileau and Hebert 1988). Further, the genetic diversity of populations formed since the last glacial maxima was reduced, and represented a subset of that available in

refugial populations, suggesting that they were likely colonized by only a few individuals (Boileau and Hebert 1991). They proposed that as populations rapidly increase in density from a few colonizers, the resultant genetic structure of each population would resemble the initial colonizers, and hence be different from nearby populations due to a sampling effect. Boileau et al. (1992) suggest that these gene frequency divergences are not at equilibrium, which violates the assumptions inherent in calculations of gene flow from divergence rates. Thus, these priority effects can drastically slow the decay rate of population genetic differentiation, despite potentially high levels of continuous gene flow. Such differentiation could take hundreds to thousands of years to decay, especially for large populations (e.g., a half life of ~7000 years with $Nm = 1$ and $N = 10^5$). Yet, over time gene flow will lead to increased genetic diversity within populations in spite of founder effects. At least for recently colonized habitats, there is evidence that such founder events and priority effects occur and lead to population genetic differentiation (Haag et al. 2006, Louette et al. 2007).

The Monopolization hypothesis (De Meester et al. 2002) builds on the PFE to explain long term divergence patterns in cyclically parthenogenetic species. Unlike obligately sexual or asexual zooplankton, cyclic parthenogens reproduce asexually for a portion of their life cycle and switch to sexual reproduction under specific conditions (Kleiven et al. 1992, Pijanowska and Stolpe 1996). This allows for rapid clonal replication of initial founders, followed by the production of sexually produced diapausing eggs. This hypothesis adds selection to the PFE, predicting that initial colonizers multiply and rapidly adapt to local environmental conditions over only a few seasons. Adaptation, in conjunction with clonal replication and annual bouts of sex, leads to the rapid development of a large diapausing egg bank that restarts the population in subsequent years. Thus, locally adapted clones are able to monopolize resources and prevent the successful invasion of new genotypes into the population. This increases population genetic differentiation by limiting the success of future colonizers (and therefore the accumulation of local diversity) and enhancing the priority effects of the initial founders. Contrary to the PFE, if selection limits gene flow among populations, genetic divergence among and the frequency of private alleles within populations should increase over time (Ishida and Taylor 2007).

Although both hypotheses seek to explain the observation of long lasting population genetic divergence through founder effects, the Monopolization hypothesis additionally suggests selection leads to local adaptation and biotic resistance to new genotypes, further reducing effective gene flow. Previous work has demonstrated local adaptation for some traits in cyclically parthenogenetic zooplankton (e.g., life history tradeoffs – Boersma et al. 1999, phototaxis – Cousyn et al. 2001, migration behavior – Michels et al. 2007), but responses to many ecological variables are plastic (e.g., temperature, salinity – Mitchell and Lampert 2000, Ortells et al. 2005). Evidence for local adaptation to resources is mixed. Research in lakes suggests zooplankton species vary in their ability to exploit rich versus poor quality resources (Desmarais and Tessier 1999, Tessier et al. 2000, Tessier and Woodruff 2002). Similar results have been demonstrated for *Daphnia* from adjacent lakes differing in food quantity and quality (Declerck et al. 2001) and in response to different food types (Sarnelle and Wilson 2005). Additionally, in competition experiments, different *Daphnia* clones have been more successful under different resource qualities (Weider et al. 2005). However, other work suggests *Daphnia* of the same species respond plastically to resource richness (Tessier and Consolatti 1991). Thus, the question remains: does resource monopolization by resident genotypes occur in small ponds?

Here, we used four populations of *Daphnia pulex* to test the predictions of the PFE and Monopolization hypotheses. From a series of previously studied ponds in central Illinois (Allen, in review), we chose four populations that differed in resource quantity and quality. We first tested whether the populations exhibited a high degree of population genetic differentiation characteristic of the PFE and Monopolization hypotheses and looked for evidence of regional gene flow using five microsatellite loci to quantify genetic diversity. We then looked for evidence of local adaptation to resources using reciprocal transplant studies.

3.3 METHODS

3.3.1 Study Organism

Daphnia pulex is a common, widely distributed cladoceran occurring in temporary ponds across the United States and Canada (Brooks 1957, Hebert 1995).

Although there is evidence for obligate parthenogenetic populations of *Daphnia* in our study region (Paland et al. 2005), our populations are cyclically parthenogenetic (M. Allen, unpublished data). In temporary ponds in Illinois, individuals emerge from a diapausing egg in winter or early spring, proceed through 2-3 generations of asexual (clonal) reproduction, and switch to sexual reproduction in late spring. Sexual reproduction results in pairs of diapausing eggs, which are encased in a desiccation-resistant ephippium and can remain dormant for years to decades (Cáceres 1998). Ephippia are the primary mechanism for dispersal among habitats or through time (Hebert 1978, Allen 2007).

3.3.2 Pond Characteristics

We chose four shallow, fishless ponds containing ephemeral populations of *Daphnia pulex* located within 50 km of one another in east-central Illinois, USA. The ponds vary in physical, chemical and biological parameters (Table 3.1). Additionally, the ponds exhibit visibly different algal and hydrophyte communities: *Lemna* mats dominate Dump Pond and filamentous algal blooms frequent Busey Pond, whereas no hydrophytes or algal blooms have been observed in Top or BridgeS ponds (M. Allen, personal observation). This suggests the resources of these ponds support different community structures and may provide different selective environments.

To identify the specific resource environment of each community, we first measured nutrients and chlorophyll a levels in each pond during mid-April 2008. Using water collected from the field for the laboratory experiment (see below), chlorophyll a (Chl-a), particulate carbon (IPCC), nitrogen (PN), phosphorus (PP), and total phosphorus (TP) were measured. Chlorophyll content was calculated by filtering pond water through a 0.7 μm filter (Whatman GFF), extracting the chlorophyll in ethanol and measuring the absorbance using a Turner Designs -700 fluorometer (Welschmeyer 1994). Pond water for TP was frozen prior to analysis. For PP/CP/NP, water was filtered through 75 μm mesh to extract large invertebrates and then through precombusted GFF filters. Phosphorus filters were frozen while nitrogen filters were stored in a dessicator until analysis. Total and particulate phosphorus were extracted by the molybdate-ascorbic acid method (APHA 1980) and analyzed using a Unico Spectrophotometer 2800. We measured particulate carbon and nitrogen on Carlo Erba NCS2500 elemental analyzer,

with acetanilide used for standards. Particulate carbon to nitrogen and phosphorus ratios were used to examine differences in resource quality among the ponds. We also measured a composite variable for resource availability using a bioassay. A juvenile growth rate assay (see detailed description below) was performed on a standard *Daphnia pulex-pulicaria* hybrid clone in each of the water types. The relative growth rate was used as an indicator of resource richness (Lampert and Trubetskova 1996, Desmarais and Tessier 1999, Tessier and Woodruff 2002).

3.3.3 Genetic Analysis

We used five polymorphic microsatellite markers to examine genetic diversity within and differentiation among our four populations. The markers were chosen from Colbourne et al.'s (2004) library of *D. pulex* microsatellite markers (Dp26, Dp156, Dp244, Dp300, and Dp335). We included *Daphnia* clones hatched from sediments in the laboratory (some of which were included in the local adaptation experiment below) and individuals from early April field samples for our estimate of the genetic composition of each spring population. Twenty-one to 23 individuals were genotyped from each population. We extracted DNA using the Qiagen DNeasy-96 tissue kit. PCR reactions used 3 μL of genomic DNA, 1 μL of 10X PCR buffer (Invitrogen), 2.5 mM $MgCl_2$, 0.2 mM of each dNTP, 0.2 μM of each primer, 1 U Taq DNA polymerase, and water to a final volume of 10 μL. We employed a "touchdown" thermocycling protocol consisting of an initial denaturation step at 94° C for 2 minutes followed by 10 cycles of: 94° C for 30 seconds (denaturation), an initial annealing temperature of 58° C for 30 seconds, decreasing by 1° C for each cycle, and an extension at 72° C for 1 minute; 30 subsequent cycles using the same denaturation and extension as above but an annealing temperature of 48° C; and followed by a final extension step of 72° C for 10 minutes (Cristescu et al. 2006). Microsatellite loci were diluted and multiplexed as unique combinations of allelic size range by fluorescent dye colors (6-FAM, HEX, VIC, PET; Applied Biosystems, Foster City, California). Genotyping was performed on an ABI 3730xl Genetic Analyzer at the University of Illinois W.M. Keck Center for Comparative and Functional Genomics.

We used the program GeneMapper 3.7 to examine and confirm allele designations for all loci in each individual. We then calculated allelic frequencies, the number of

alleles per locus (*A*), expected heterozygosity (H_E), the total number of private alleles (*PVT*) and unique multilocus genotypes (*MLG*) within each population to determine estimates of genetic diversity and make inferences about regional gene flow. To test for population divergence, the total genetic variation among and within each of the populations was partitioned using AMOVA (Excoffier et al. 1992) and estimates of F_{ST} analogs (θ: Weir and Cockerham 1984) for pairwise combinations of populations were calculated. We used a sequential Bonferroni correction for multiple comparisons for differentiation tests, where appropriate (Rice 1989). All analyses were performed in Arlequin 3.1 (Excoffier et al. 2005).

3.3.4 Local Adaptation Experiment

To test for local adaptation to resources, we designed an experiment in which clones from each population were reciprocally grown in water from each pond in a laboratory common garden. We used the juvenile growth rate assay (Lampert and Trubetskova 1996, Desmarais and Tessier 1999, Tessier and Woodruff 2002) to measure the growth rate of each clone on each water source. We also measured resource richness using the resource bioassay described above. We then tested for local adaptation following Kawecki and Ebert (2004). If populations are locally adapted to resources, then native clones should have higher average growth rate than foreign clones for each water source.

To ensure that the experiment used a set of unique clones that were representative of early spring population colonizers (and hence to test the Monopolization hypothesis assumption of a locally adapted egg bank), we hatched *Daphnia* from diapausing eggs collected from sediments. During fall 2007, sediment containing *Daphnia* ephippia was collected from each of the dry ponds and brought to the laboratory where it was stored at 5°C for three months. In January 2008, the sediment was incubated in filtered lake water in the laboratory under early spring-like conditions. Containers were checked twice per week for two months for hatching. All hatched *Daphnia* were immediately transferred to 150 ml beakers and maintained in standard laboratory culture (15°C, 12:12 dark/light cycle, fed *Ankistrodesmus falcatus*).

Eight distinct clonal lines from each pond were selected randomly for use in the reciprocal transplant experiment. Clones were split into multiple sublines and grown in

low density culture for two generations to reduce maternal effects following Lynch (1985). Each new generation was started with neonates from the third or greater clutch. Clones were kept at 20°C in a 12:12 dark/light cycle and fed a satiating amount of *Ankistrodesmus* algae daily. Neonates from the third clutch or greater from the second generation of each line were used as experimental animals for the reciprocal transplant experiment.

To measure the relative fitness of each clone on all four resource types, we performed a juvenile growth rate (JGR) assay. The JGR is the four day somatic growth rate for a daphniid clone. It is a standard fitness measure for daphniid species, and been shown to be a very reliable indicator of total lifetime reproductive output (Lampert and Trubetskova 1996, Tessier and Woodruff 2002). Using a 4 x 4 factorial design, we grew eight *Daphnia* clones per population, with two replicates per clone in each water type. Due to logistical constraints, we blocked the experiment over six consecutive days with two water types per clone started on each day. The order of the four water resources was randomized for each clone. To start the experiment, neonates from a single mother's third or greater clutch were gathered within 18 hours of expulsion from the brood pouch. Five sisters from each maternal clone were harvested, dried in a drying oven at 60°C and weighed on a UMX2 microbalance (Mettler Toledo) to get a measure of initial weight. Five additional sisters from each maternal clone were placed in a 200 ml beaker with the designated water resource and grown for four days. Water was filtered through 70 μm mesh to remove invertebrates and changed daily to replenish resources. On day three densities in each beaker were reduced to two individuals. On day five, all individuals were harvested, dried at 60°C and weighed. JGR was calculated for each clone as the log of the average weight at the end of the experiment minus the log of the initial weight divided by the number of days grown.

We followed the methods suggested by Kawecki and Ebert (2004) to test for adaptation to local resources. We used a two-way ANOVA with water source (S) and clone source (population - P) as fixed factors. Clones were nested within population as a random factor. A four degree of freedom planned contrast was used to test for local adaptation: clones grown on their local resources versus foreign clones grown on those same resources. The analysis was run using the Type 3 least squares approach with a

Satterthwaite degrees of freedom correction. Due to insufficient reproduction of clonal mothers and dying of some experimental animals during the experiment, we were not able to replicate fully the 4 x 4 factorial experiment. As such, for each clone by resource growth estimate we used the average of the available sublines as our experimental unit to test for local adaptation. Clones for which we had growth estimates in their own water and at least one foreign water source were included (BridgeS: 7 clones, Busey: 7, Dump: 5, Top: 5; Appendix H). Post hoc power analyses suggested these sample sizes were sufficient to achieve $(1-\beta) > 0.9$ for both main effects and their interaction (Faul et al. 2007). Running the analysis as a fully balanced design by eliminating clones with growth estimates in only two or three water types did not qualitatively change results.

As we had no estimates for intraclonal variation using the above statistical analysis, we used a subset of the data to elucidate further mechanism(s) underlying clonal growth responses in variable environments. We tested for clonal plasticity or adaptation to resources using only those clones which had multiple replicate estimates in at least two of the four habitat types (15 clones). We tested for plasticity to resource richness using a slope homogeneity test, using our aggregate measure of resource availability as a covariate crossed with clone (a random effect). Heterogeneous slopes among the clones would suggest a G x E response indicative of a genetic effect on clonal plasticity. We then performed ANCOVA to test for genetic variability among clones using this same covariate. As there were only 15 clones, we did not test for an effect of host population on the growth response. All analyses were run in SAS 9.1 (SAS Institute, Cary, NC).

3.4 RESULTS

3.4.1 Pond Resources

The ponds varied substantially in size, hydroperiod and trophic status (Table 3.1). Differences in resources were reflected by significant variation in food quantity as measured by chlorophyll a concentration in the ponds ($F_{3, 8} = 42.03$, $p < 0.0001$). Food quality was similar among the ponds, as there were no significant differences in the C:N ratio ($F_{3, 3} = 0.40$, $p = 0.77$) or C:P ratio ($F_{3, 3} = 0.18$, $p = 0.90$) (the latter due to large standard errors). These general trends were reflected in our bioassay of resource use, as BridgeS Pond had the highest quantity of food and most effective conversion rate to

growth, while Busey and Dump ponds had lower quantities of food and the lowest growth rates (Table 3.1). However, there was not a linear relationship between individual measures of quantity and quality (i.e., chlorophyll or C:P) and the composite resource richness bioassay (chl-a: r = 0.75, p = 0.25; C:P: r = -0.26, p = 0.74).

3.4.2 Microsatellite Diversity

Ninety individuals from the four populations were genotyped over five loci, 44 of which were hatched directly from diapausing eggs. Consistent with the expectations of the PFE and Monopolization hypotheses, there was significant population genetic differentiation among all populations. All pairwise F_{ST} estimates were significant despite the geographic proximity of the populations (mean [SE] F_{ST} = 0.21 [0.05]; Table 3.2). Even Top and Dump ponds, which are separated by only 250 m showed moderate and significant differentiation (F_{ST} = 0.10). Overall, AMOVA indicated that nearly 22% of the total variation was divided among populations, while only 10% was among individuals within populations (most was within individuals).

Consistent with ongoing gene flow (hence, contrary to the Monopolization hypothesis), many alleles were shared among populations and regional genetic variation was high (mean H_E = 0.62). All loci were polymorphic with 4 to 14 unique alleles per locus and at least three alleles were found for each locus in each population, with a maximum of 12 alleles found (Dump Pond - Dp26). High allelic diversity was seen in mean expected heterozygosities within each population (range: 0.457 – 0.736; Table 3.3). The high percentages of unique multilocus genotypes found within and among populations also reflected the large regional variation. Additionally, there was a low frequency of private alleles in the populations and most were resident in the most genetically diverse populations, indicative of some dispersal across a regional gene pool (Table 3.3).

3.4.3 Local Adaptation

We found no evidence for local adaptation to resources in our reciprocal transplant experiment (Fig. 3.1). The mean JGR of clones grown in their home water did not significantly exceed that of foreign clones for any of the four ponds (contrast: $F_{4, 54}$ = 0.53, p = 0.71, Table 3.4). Likewise, there were no systematic differences in mean JGR among or within the populations. However, there was a large effect of environment (S)

on the mean growth of clones across the four habitats (Table 3.4). At the clonal level, we found no evidence for differences in phenotypic plasticity among the clones (slope homogeneity test: $F_{14, 89} = 0.67$, $p = 0.80$) or genetic variability in growth rate across a resource gradient (ANCOVA: $F_{14, 89} = 1.00$, $p = 0.46$). However, we found evidence for an effect of resource richness on growth (ANCOVA: $\beta_Q = 0.71$, $F_{1, 103} = 82.77$, $p < 0.0001$; Fig. 3.2). This suggests clones from these temporary ponds have a strongly plastic response to resource richness, but little genetic differentiation in this response.

3.5 DISCUSSION

The Monopolization hypothesis predicts that substantial genetic differentiation in populations of cyclical parthenogens results from a combination of persistent founder effects and rapid adaptation of newly founded populations to local environmental conditions (De Meester et al. 2002). Our population genetic results mirror other studies on cyclic parthenogens that provide strong support for genetic differentiation and persistent founder effects among geographically proximate ponds (Boileau and Hebert 1988, Spitze 1993, Vanoverbeke and De Meester 1997, Lynch et al. 1999, Morgan et al. 2001, Gomez et al. 2002, Haag et al. 2006). However, in contrast to predictions from the Monopolization hypothesis, we found high genetic diversity within populations, a majority of alleles shared across the region, and no evidence for local adaptation of relative growth rate to divergent resource conditions. Hence, while local adaptation to resources and reduced gene flow may play an important role in maintaining founding effects in other systems, our results suggest that it does not contribute to an explanation of patterns of persistent founder effects in this system.

Consistent with both hypotheses, genetic differentiation among the four *Daphnia pulex* populations was moderate to high, according to Wright's (1978) guidelines for interpretation of F_{ST} values. Boileau et al.'s (1992) simulations showed that for the level of genetic divergence observed among our populations, only one to five individuals likely founded the populations. Assuming rapid population growth to carrying capacity as in their model, the elimination of allele frequency differences among populations through migration and drift alone would take thousands of years, even with high gene flow ($Nm = 5$) and small (10^5) population sizes. Because our *Daphnia* populations are relatively

young (e.g., Top Pond is a shallow drainage basin of an old railroad bed), we expected some genetic divergence as a result of persistent founder effects and a lack of drift-migration equilibrium among systems.

However, if local adaptation is preventing the successful colonization of new genotypes into a system as predicted by the Monopolization hypothesis, one would expect increased genetic differentiation and higher frequencies of private alleles over long time periods (Ishida and Taylor 2007). We observed high genetic diversity in the populations (e.g., BridgeS $H_E = 0.74$), high allele sharing among populations, and a low frequency of private alleles (and they were mostly rare). These results suggest that, despite obvious founder effects, populations have been colonized by many individuals over time, and that gene flow occurring at the regional level reduces persistent founder effects through time, propositions not consistent with the Monopolization hypothesis. Other work has also shown new genotypes can effectively colonize new *Daphnia* populations (Haag et al. 2005, Louette et al. 2007). Immigrants may actually be favored in genetically depauperate populations, as they can reduce inbreeding depression and can experience hybrid vigor after sexual reproduction with population inhabitants (Ebert et al. 2002). Although these data suggest gene flow occurs, a temporal record of genetic diversity changes is needed to effectively test this hypothesis.

Because our experiments used *Daphnia* hatched from the egg bank or before rapid clonal reproduction in these ponds, we can effectively rule out seasonal clonal selection as one cause of population genetic differentiation. *Daphnia* populations often adhere to Hardy-Weinberg expectations shortly after hatching from sexual eggs (Lynch et al. 1989, Innes 1991), but as populations proceed through a season, selection for specific clones may take place altering genotypic frequencies (Hebert 1974, Young 1979). Across multiple systems, different clonal lineages will be selected during a season, increasing the F_{ST} among the populations. Of the four populations, Busey Pond was the most likely population to experience significant bouts of clonal selection, because the population exists year round and the pond only dries every few years (Lynch 1984a; M. Allen, unpublished data). However, by using individuals hatched directly from sediment or water column early in the season, we took advantage of the recent bout of sex and avoided allowing the immediate effects of clonal reproduction to affect our analyses.

Additionally, avoiding intraseason clonal selection was important for our local adaptation study, because it allowed us to assess long term consequences of evolution on the population as opposed to seasonal effects.

3.5.1 Resource Plasticity and Local Adaptation

We found no evidence for local adaptations to resources in our four populations. Additionally, individual clones from each of the habitats had similar juvenile growth rate responses across the range of resources, exhibiting plasticity to resource richness. Resource plasticity may result from the selection of broadly adapted, plastic genotypes over individuals with specialized responses. Generalist genotypes may be favored where individuals experience multiple environments over the course of their lifetime. If dispersal is very high, generalists may be selected where genotypes experience different habitats over the life of a clone. Alternatively, generalists may be selected where temporal environmental variability is a stronger selective force than spatial variability (Reboud and Bell 1997, Kassen 2002). In both lakes and ponds, variation in resource quality and quantity over the length of a growing season can be substantial (e.g., Declerck et al. 2001, Cáceres et al. 2008). Responding to such variation over the lifespan of one clone may require differential growth or life history responses. Resource variability, specifically, has been shown to elicit plasticity in a number of life history traits (e.g., size of offspring – Tessier and Consolatti 1991, resource allocation – Stelzer 2001). Thus, responding plastically to such variability would provide the most flexible fitness response through uncertain conditions.

Although we found no evidence for local adaptation to resources here, Declerck et al. (2001) showed that *Daphnia* clones from one lake experienced reduced survival relative to those from another lake when grown on water from the second. They concluded clones from each lake were adapted to their natal environment. While local adaptation may have occurred in these lakes, a lack of evidence for it in our ponds may have resulted for two reasons. First, in Declerck et al.'s (2001) study one lake had consistent high food quantity levels (> 100 µg/L chlorophyll-a), whereas the other had lower food quantity levels that varied by greater than an order of magnitude during the year (2 – ~50 µg/L chlorophyll-a). This difference in the magnitude of and seasonal variation in food availability may cause strong selective differences among systems, and

we have no data on such temporal patterns in our systems. Second, clonal selection may have stronger effects in lakes relative to ephemeral ponds as the growing season is longer, and some populations can exist perennially (Lynch 1983). When many generations exist between hatching and sexual reproduction, selection for clones best suited to local resources may be more efficient as less suited clones fail to engage in sexual reproduction. This effect is much reduced in ponds with few clonal generations.

Alternatively, selective forces other than those related to resources – such as the chemical conditions, composition and abundance of interspecific competitors, predators or pond permanence – may lead to local adaptation and the population genetic consequences consistent with the Monopolization hypothesis. Prior work with *Daphnia* has demonstrated selection for different chemical tolerances (Weider and Hebert 1987), on behavior (e.g., De Meester 1996, Michels et al. 2007), as well as numerous traits that vary in response to invertebrate and vertebrate predation pressures (e.g., diapause timing shifts in response to fish predation - Hairston and Dillon 1990, neck tooth development in response to invertebrate predators - Parejko and Dodson 1991, body size evolution - Tessier et al. 1992, Boersma et al. 1999, phototaxis - Cousyn et al. 2001). Although a number of these factors are not pertinent to our systems (e.g., fish are absent), interspecific competition and invertebrate predators are common in temporary ponds. Competition for food has been shown to affect the life history traits of populations and composition of communities (Lynch 1978, Bengtsson 1986, Nandini et al. 2002, Milbrink et al. 2003). Whereas *D. pulex* is the dominant taxa for Dump and Top ponds throughout the spring, Busey and BridgeS ponds are dominated by other daphniid taxa early in the season (*Ceriodaphnia* and *Daphnia ephemeralis*, respectively). Such community changes may affect population phenology and resources available to the organisms. Additionally, while invertebrate predators are infrequent during the exponential growth phase of *Daphnia*, their presence during sexual reproduction may also cause differential selective effects across habitats (Pastorok 1981, Riessen 1999). We subsequently explored this possibility by measuring the body size of ephippial *Daphnia* during their peak abundance in the water column. Body size distributions significantly differed among the populations. Additionally, the peak ephippial production window varied among populations by over of a month (M. Allen, unpublished data). Such observations may indicate an effect of

invertebrate predators or competition on size distributions and phenology, but whether these observations are a locally adaptive response or simply another example of phenotypic plasticity is currently unknown. If such effects were to increase the survival and fecundity of local organisms over immigrants, they may contribute to reduced gene flow and the maintenance of population genetic diversification among populations as predicted by the Monopolization hypothesis.

3.5.2 Conclusions

Our *Daphnia* populations exhibit substantial genetic differentiation despite their close geographic proximity and high degree of genetic diversity. The Monopolization hypothesis stresses the importance of rapid local adaptation of early founders for maintaining and augmenting persistent founder effects. Here, we have demonstrated that it is unlikely that our population differentiation results from local adaptation of founders to local resource availability. Instead, our results suggest gene flow has persisted since population founding and growth plasticity may allow immigrant clones to successfully colonize and persist in habitats with a variety of resource conditions. Alternatively, if the monopolization of resources through local adaptation does influence early colonization success, it rapidly becomes unimportant as clonal diversity increases through sex and subsequent immigration.

3.6 ACKNOWLEDGEMENTS

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3.8 TABLES

Table 3.1

Biotic and abiotic characteristics of Illinois study ponds in April 2008. Variables: *Size* (m^2) as measured from aerial photos in ArcGIS Desktop. Maximum *depth* in meters. *Hydroperiod* given in months: 12+ = pond fails to dry in wet years, sp = semipermanent, rarely dries. *Resources*: juvenile growth rate from *D. pulex-pulicaria* bioassay. Chlorophyll-a concentration (*Chl-a*) and total phosphorus (*TP*) in µg/L. *C:P (C:N)*: seston carbon to phosphorus (nitrogen) ratio was calculated for each date and the average calculated. Standard errors are given, where available.

Pond	Location	Size (m^2)	Depth (m)	Hydroperiod
BridgeS	40.1221 N, 97.7367 W	2230	1.5	7 – 12+
Busey	40.1287 N, 88.2131 W	12150	1	8 – 12+
Dump	40.2428 N, 87.7795 W	270	1.5	sp
Top	40.2420 N, 87.7824 W	290	0.5	3 – 6

Pond	Resources	Chl-a (µg/L)	TP (µg/L)	C:P	C:N
BridgeS	0.504	4.60 (0.48)	203.4	69.6	8.9
Busey	0.269 (0.04)	1.60 (0.24)	21	128.9 (75.7)	8.2 (0.6)
Dump	0.234 (0.05)	0.75 (0.22)	48.4	65.1 (7.2)	7.4 (0.6)
Top	0.386	0.49 (0.06)	30.1	104.6 (89.6)	9.0 (1.8)

Table 3.2

Population pairwise genetic divergence (F_{ST}) values. A global test for population genetic differentiation revealed significant pairwise differentiation ($F_{ST} = 0.216$). All pairwise comparisons were significant at the $p < 0.0001$ level using a permutation test.

	BridgeS	Busey	Dump	Top
BridgeS				
Busey	0.253			
Dump	0.070	0.290		
Top	0.159	0.405	0.101	

Table 3.3

Genetic diversity of *Daphnia pulex* populations using five polymorphic microsatellite loci. Variables: Number of genotyped individuals – hatched from eggs/total (*n*). Average number of alleles per locus (*A*). Expected heterozygosity (H_E). Number of private alleles (*PVT*). Percent unique multilocus genotypes in the population (*MLG*). Diversity estimates are mean (± SE) by locus.

Pond	***n***	***A***	***HE***	***PVT***	***MLG***
BridgeS	15/23	6.4 (1.3)	0.736 (0.056)	5	22/23 = 95.7
Busey	15/23	3.8 (0.58)	0.457 (0.102)	0	20/23 = 87.0
Dump	7/23	6.6 (1.5)	0.714 (0.059)	3	22/23 = 95.7
Top	7/21	4.2 (0.37)	0.574 (0.090)	1	21/21 = 100.0
Total	44/90	5.25 (0.82)	0.620 (0.049)	9 (43 total)	84/90 = 93.3

Table 3.4

Analysis of variance testing for the effects of water source (S) and population (P) on the relative fitness of *Daphnia.* The contrast tests the local versus foreign interaction for local adaptation.

Effect	**df effect**	**df error**	**Mean Square**	**F**	**p**
S	3	20.98	0.1992	33.45	<0.0001
P	3	54	0.0050	0.54	0.66
S*P	9	54	0.0055	0.92	0.51
Clone(P)	20	54	0.0094	1.57	0.10
Residual	54		0.0060		
Contrast					
Local vs. Foreign	4	54		0.53	0.71

3.9 FIGURES

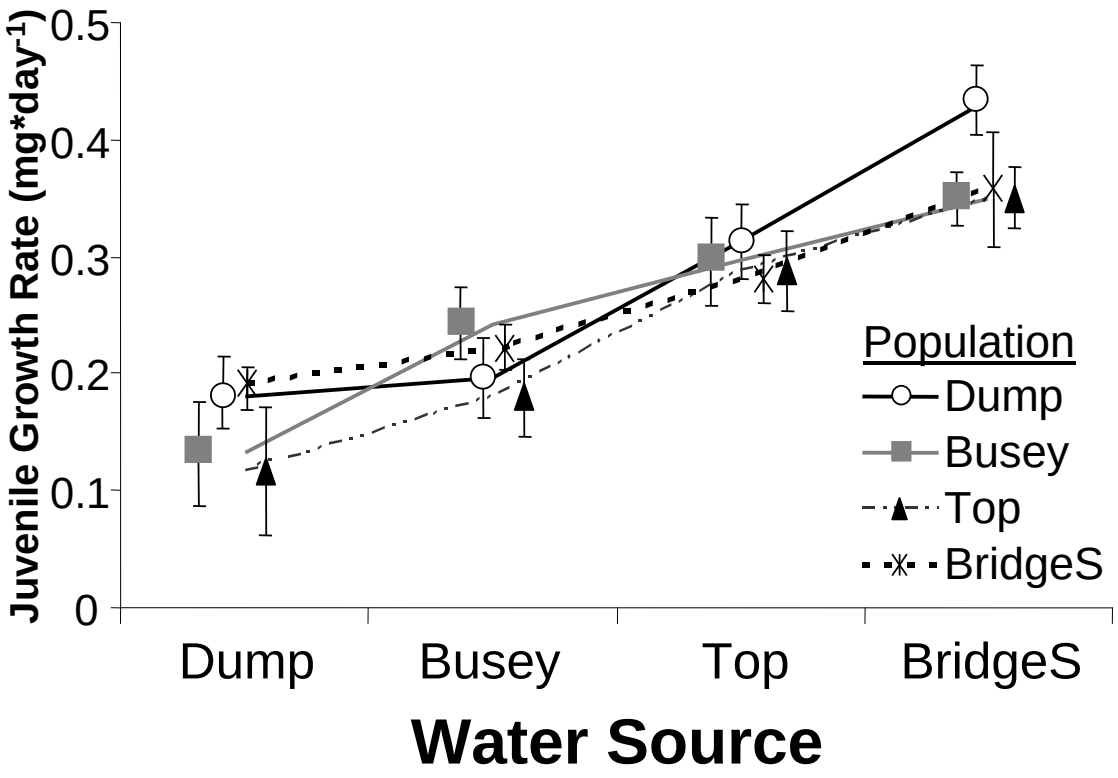

Figure 3.1

Mean relative fitness within four *Daphnia* populations grown on four host resources. Relative fitness was measured as the four day somatic juvenile growth rate. Each set of points represents clones from a single source population. Host ponds are ordered from the lowest to highest relative resource abundance (from bioassay). There was a strong effect of water source, but no effect of population or clone on relative growth. Error bars represent standard error and reflect among-clone variation.

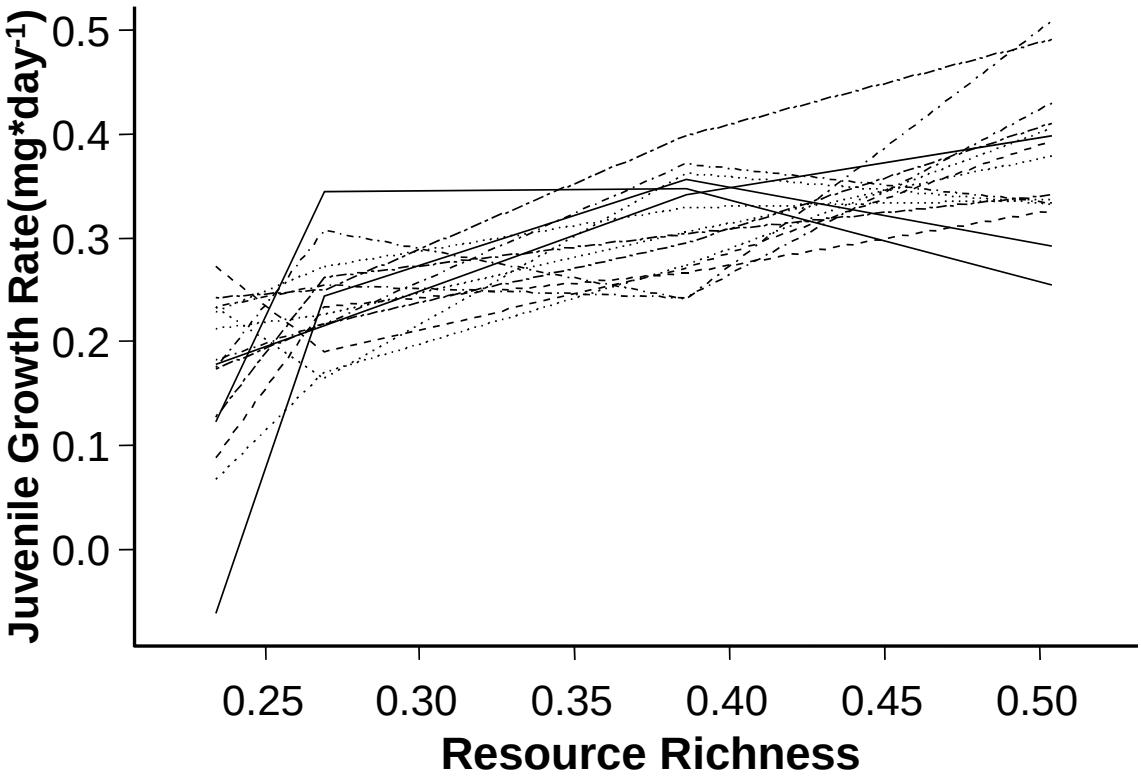

Figure 3.2

Mean juvenile growth rate of *Daphnia* clones in four resource environments. Resource richness was calculated by the *D. pulex-pulicaria* resource bioassay (mg*day^{-1}). Clones hatched from each of the four habitats were included in the analysis. There was a strong effect of resource richness, but no effect of clone or its interaction with environment. Resource richness from left to right: Dump Pond, Busey Pond, Top Pond, and BridgeS Pond. For clarity, error bars have been removed.

Chapter 4: Evolutionary-community ecology: Zooplankton diversity across multiple levels of biological diversity

4.1 ABSTRACT

By focusing on dispersal, metacommunity theory is a promising approach for integrating community ecology with evolutionary biology, as dispersal concurrently affects both ecological and evolutionary dynamics. We studied the zooplankton assemblages in a series of recently formed lakes to examine how ongoing dispersal has affected community, population, and genetic composition in the region. Despite differing colonization sequences, current cladoceran composition was sorted into groups which clustered by lake depth. Given species sorting at the community level, we asked whether phenotypic and genetic variation were structured similarly. Body size distributions of the two largest species also differed among lakes, although only *Daphnia pulicaria* body sizes sorted by lake depth in a common garden. However, total quantitative trait differentiation was low (Q_{ST}: *D. pulicaria* = 0.13, *D. dentifera* = 0.11), and much of the variation resided within lakes, indicating the long term maintenance of variation through temporal or spatial dispersal. A considerable portion of the molecular genetic variation was distributed among lakes (mean F_{ST}: *D. pulicaria* = 0.13, *D. dentifera* = 0.21). However, this variation appeared to diverge neutrally among populations and independently of the sorting operating at the level of phenotype and community, as there was no relationship between neutral genetic variation and community clusters or pairwise quantitative genetic differentiation. Additionally, there was some evidence for neutral genetic isolation by distance among *D. pulicaria* populations. Thus, while it is apparent that dispersal can result in structure at multiple levels of biological diversity, the underlying cause (i.e., selection versus drift) at any particular scale may be different.

4.2 INTRODUCTION

Traditionally, evolutionary biology and community ecology largely have been studied separately, as the processes studied in each sub-discipline were considered to occur on separate timescales (e.g., Thompson 1998). However, as observations of rapid evolution mount, researchers recognize that such dichotomies hamper our ability to understand the complexities of ecological dynamics (Thompson 1998, Hanski and

Gaggiotti 2004, Hairston et al. 2005, Johnson and Stinchcombe 2007). Increasingly, studies of community assembly, interspecific interactions, life history variation and population and community genetics show that incorporating evolutionary interactions is necessary to understand observed patterns (Chase and Leibold 2003, Kawecki and Ebert 2004, Ronce and Olivieri 2004, Thompson 2005, Emerson and Gillespie 2008, Urban et al. 2008). Integrative studies examining variation at multiple levels of biological diversity (species, trait, neutral genetic) are needed to understand better the interaction between contemporary evolution and patterns of diversity in nature (Stockwell et al. 2003).

Metacommunity theory is a promising conceptual framework for integrating the effects of connectivity and local processes at multiple levels of biological diversity (Leibold and Norberg 2004, Urban and Skelly 2006, Loeuille and Leibold 2008, Urban et al. 2008). Specifically, this theory considers how connections between habitats (i.e. dispersal) influence the ecology of and evolution in local communities (Gilpin and Hanski 1991, Holyoak et al. 2005). Where dispersal is moderate or low, community structure may be influenced by chance colonization-extinction events (neutral or patch dynamics perspectives). In the neutral case, species are considered to be competitively equivalent, and local microevolution acts through drift (Hubbell 2001). Under patch dynamics, species interactions and dispersal influence community structure. Evolution can occur in response to interspecific interactions. At moderate levels of dispersal in a geographically structured landscape, species can reach all habitats, but environmental gradients determine community structure (Whittaker 1972, Leibold et al. 2004). High dispersal, alternatively, can homogenize local community composition, in spite of geographic structure, and can overwhelm any local adaptation due to the constant mixing of regional genotypes (mass effects: Mouquet and Loreau 2002, Leibold et al. 2004, Holyoak et al. 2005). Thus, the mechanisms structuring local composition in a community context depend on both the relative influence of connectivity and habitat structure (Leibold et al. 2005, Urban et al. 2008).

Dispersal will likewise shape the distribution of trait values and genetic variation of individual species within a metacommunity. At high levels of dispersal where mass effects homogenize community composition, one also expects populations to exhibit similar trait values regardless of the local habitat conditions due to immigration of

regional genotypes and similar interspecific interactions among habitats (Leibold and Norberg 2004). Similarly, neutral genetic variation may be homogenized across the metacommunity (Slatkin 1985). With lower immigration, trait and genetic distributions may be shaped by local adaptation or by drift. To some extent, we expect local distributions within any habitat to be a subset of the total regional variation due to sampling effects. But the degree to which this is the case depends on the magnitude of local and regional influences on the local community. At this point, predictions of metacommunity effects on trait and genetic distributions are mostly theoretical, and little has been done applying them to natural systems (but see Urban 2004).

Zooplankton assemblages have been used to test metacommunity theory for a number of years (Cottenie et al. 2003, Cottenie and De Meester 2004, Jenkins 2006, Vanschoenwinkel et al. 2007, Altermatt et al. 2008, Howeth and Leibold 2008). Despite early assumptions that lakes and ponds were isolated habitats, recent work has linked local communities with regional processes, and shown overland transport of zooplankton to be a regular process (Cáceres and Soluk 2002, Cohen and Shurin 2003, Cottenie et al. 2003, Allen 2007, Vanschoenwinkel et al. 2008). Some have suggested that movement is at least substantial enough that dispersal is not limiting in the colonization of habitats (Shurin 2000, Louette and De Meester 2005). However, high rates of genetic differentiation among populations suggest the success of dispersal after initial colonization may be minimal (De Meester et al. 2002, Gomez et al. 2002). Yet, dispersal may be ongoing as it may take thousands of generations to eliminate observed founder effects (i.e., populations are not at genetic equilibrium) due to clonality and large populations sizes of many zooplankton (Boileau et al. 1992, Allen Chapter 3). As many zooplankton taxa make dormant stages that can remain viable for tens or hundreds of years (Hairston et al. 1995, Cáceres 1998), they are good candidates for studying the influence of dispersal over space and time on community, trait and genetic variation. Documenting variation at these multiple levels of biological diversity will contribute to understanding the effects of dispersal on metacommunity evolution.

In this study, we investigate if a particular level of ongoing dispersal in a metacommunity structures different levels of biological diversity (community/species, trait, neutral genetic) in the same way. To address this, we studied cladoceran

communities in eight recently formed lakes in central Illinois. At the species level, colonization history and current community composition suggest that communities are structured by species sorting processes. Given this result, we used two species of *Daphnia* to test whether an ecologically important trait (body size) showed ecological sorting of phenotypes in the field, and if there was quantitative genetic differentiation for this trait among the populations. We specifically tested whether dispersal limitation influenced differentiation of quantitative traits, or if quantitative differentiation reflected the same sorting pattern as at the community level. Next, we asked whether neutral genetic variation showed evidence for differentiation among populations. We tested the hypotheses that 1) dispersal limitation did not influence population genetic differentiation across space, and 2) genetic diversity was a function of time since colonization. Finally, we tested whether ecologically divergent traits showed similar patterns of divergence at the genetic level, or if these levels of biological diversity act independently of one another.

4.3 METHODS

4.3.1 Site History and Study Organisms

Our eight study lakes are located in Kickapoo State Park (Vermilion Co, IL, USA) (Figure I.1). Strip mining for coal began in 1863. Once mining ceased, the newly-created pits became spring fed lakes of similar size and shape to natural glacial kettles. This created a series of habitats that vary in surface area, depth and age between 1926 and 1959 (Table J.1). Since stratified lakes are not a common feature of the east-central Illinois landscape, these lakes provided a novel habitat for pelagic zooplankton in the region. Once filled, the lakes were stocked with largemouth bass (*Micropterus salmoides*), bluegill sunfish (*Lepomis macrochirus*) and various other fish species (Horner and Brummett 1972, R.W. Larimore, personal communication). Many other invertebrates, amphibians and macrophytes have also colonized these lakes. Here, we focus on the open-water cladoceran zooplankton assemblage, and specifically, the two largest-bodied species *Daphnia pulicaria* and *D. dentifera*.

4.3.2 Field Patterns

We used diapausing egg cases (ephippia) to determine the colonization sequences of cladocera into the eight lakes. Replicate sediment cores (6.5 cm inner diameter) were taken from each lake by SCUBA with a hand-held corer in May 2003 and June 2004. Cores from all lakes except Emerald reached to the sediment base which represents the date of lake formation. Cores were sliced in 1cm intervals according to the protocol outlined in Cáceres (1998) and all ephippia were removed from the sediment with a combination of sieving and density centrifugation. Ephippia in each sediment layer were enumerated and identified to species. To calculate the year of colonization for each species, we assumed an equal sedimentation rate through time to the base of the core. The approximate date of the first slice containing ephippia from a given species was recorded as the colonization date.

To determine current cladoceran species diversity, we used an 80 μm Wisconsin bucket net to collect whole-water column zooplankton samples in May 2007. Samples were preserved in 95% ethanol and all cladocerans were identified to family (chydoridae) or species (*Daphnia*, *Ceriodaphnia, Bosmina*). We used species abundance (number L^{-1}) to calculate the Bray-Curtis dissimilarity among the communities (Bray and Curtis 1957). Data were square root transformed to standardize the skewed abundances. We then used the program Cluster (Brzustowski 2002)[1] to group similar community structures using complete linkage on the matrix of dissimilarity values. Node stability was assessed with 1000 bootstrap iterations of the dataset. A matrix of minimum Euclidian distances among lakes was also measured in ArcGIS Desktop 9.2 (ESRI, Redlands, CA). We used Mantel tests to calculate the relationship between community similarity and geographic distance among sites using IBDWS 3.15 (Jensen et al. 2005)[2].

To quantify within and among lake variation in a key life-history trait (body size), we collected live *D. pulicaria* and *D. dentifera* from the six lakes that contained both species. We chose body size as our quantitative trait of interest, because it has an important relationship with fitness and it can be selected by a variety of ecological factors operating at the community level (e.g., resource quality, competition, parasites, predation

[1] http://www2.biology.ualberta.ca/jbrzusto/cluster.php
[2] http://ibdws.sdsu.edu/~ibdws/

– Norberg 2004). For each lake, fifty adult females (those with eggs, ephippia or a distended brood pouch) from both species were measured under the microscope using Spot photography software (Diagnostic Instruments, Sterling Heights, MI). We used PROC MIXED in SAS 9.2 (SAS Institute, Cary, NC) with lake as a fixed effect to compute the ANOVA and used a contrast statement to test the hypothesis that mean body size differed between lake communities grouped by cluster using restricted maximum likelihood (REML). We would expect such a pattern if ecological traits sort along a similar gradient as species composition. ANOVA with lake as a random effect was used to calculate variance components. Each species was analyzed separately.

We also quantified among-lake differences in resources levels in the epilimnion (upper water layer) with a standard bioassay (Desmarais and Tessier 1999, Tessier et al. 2000) to test whether resource richness affected the size distribution of either species. Individuals of a single clone of *Daphnia pulex-pulicaria* were grown from birth to day-4 on water collected daily from the top 3 m of each lake during the quantitative trait assay. We measured the dry weight of a number of individuals at birth and after four days, and the average daily weight change provided a relative measure of the resource availability in each system. Pearson's correlation was used to test the hypothesis that average body size of adults in the field reflected epilimnetic resource levels.

4.3.3 Common Garden Experiment

Although field patterns can reveal differences in the distribution of body sizes among habitats that may capture the influence of clonal selection and gene flow, they are also influenced by age structure (*Daphnia* have indeterminate growth), resource levels, and/or phenotypic plasticity. Thus, we quantified genetic differentiation in body size among lakes by measuring the size at maturity of individuals grown in a laboratory common garden. In May 2007, 25 iso-female lines (hereafter "clones") from six *D. dentifera* and five *D. pulicaria* populations were brought into laboratory culture. Clones were grown for three generations in environmental chambers set to a 10 dark : 14 light cycle at 20°C and fed 2 mg C L^{-1} of the green alga *Ankistrodesmus falcatus* daily to standardize maternal and grandmaternal effects (Lynch 1985). After the third generation, clones were split into two sublines and neonates from the third clutch or later were used as experimental animals. For each subline, we collected three neonates (< 18 hours)

which were placed into 200 ml GF/F filtered lake water, incubated at 10 dark :14 light cycle at 20°C, and fed 2 mg C L^{-1} of the green alga *Ankistrodesmus falcatus* daily. Experimental animals were monitored daily until production of their first clutch of eggs, at which point the animal was measured. Sizes of all females that survived to maturity in each beaker were averaged, resulting in two estimates of size at maturity per clone (one from each subline). Because of incubator space constraints, the experiment was blocked into five groups of five clones per population and blocks were run sequentially during the late summer and fall 2007. In all, 95 clones from five *D. pulicaria* populations and 117 clones from six *D. dentifera* populations were assayed.

For each species, differences among populations in mean size at maturity were determined via mixed model ANOVA with lakes as a fixed effect and clones nested within lakes as random factors. The effect of experimental block was not significant, so it was not included in any analyses. We then tested whether this life history trait grouped by lake cluster as above. Variance components were calculated using a random effects model with REML. To ascertain if phenotypic variation had a heritable component, broad sense heritabilities were calculated for each population using the variance components of one way ANOVAs fit with REML (Lynch and Walsh 1998).

To estimate quantitative trait differentiation of each species at the regional scale, we calculated the mean Q_{ST} among all populations. We used ANOVA to estimate the variance components for among lake (σ^2_A) and within lake (clonal - σ^2_W) variation using 1000 bootstraps of the whole dataset, resampling across clones within lakes. Q_{ST} was then calculated as $\sigma^2_A/(\sigma^2_A + 2* \sigma^2_W)$ following Spitze (1993), and the bootstrapped distribution was used for hypothesis testing. Bootstrap analyses were performed using the 'lmer' package in R 2.7.1 (R Core Development Team, 2008).

4.3.4 Microsatellite Methods

To quantify within and among lake molecular genetic variation, we genotyped the experimental clones from each of the *Daphnia* populations included in the common garden experiment. We extracted DNA using the Qiagen DNeasy-96 tissue kit and then genotyped polymorphic microsatellite loci using primers from Colbourne et al. (2004) and Fox (2004) (*D. dentifera*: Dgm105, Dgm 106, Dgm107, Dgm113; *D. pulicaria*: Dp208, Dp231, Dp291, Dp304, Dp339). PCR reactions used 3 μL of genomic DNA, 1

μL of 10X PCR buffer (Invitrogen), 2.5 mM $MgCl_2$, 0.2 mM of each dNTP, 0.2 μM of each primer, 1 U Taq DNA polymerase, and water to a final volume of 10 μL. We employed a "touchdown" thermocycling protocol consisting of an initial denaturation step at 94° C for 2 minutes followed by 10 cycles of: 94° C for 30 seconds (denaturation), an initial annealing temperature of 58° C for 30 seconds, decreasing by 1° C for each cycle, and an extension at 72° C for 1 minute; 30 subsequent cycles using the same denaturation and extension as above but an annealing temperature of 48° C; and followed by a final extension step of 72° C for 10 minutes (Cristescu et al. 2006). Microsatellite loci were diluted and multiplexed as unique combinations of allelic size range by fluorescent dye colors (6-FAM, HEX, VIC, PET; Applied Biosystems, Foster City, California) and run on an ABI 3730xl Genetic Analyzer at the University of Illinois W.M. Keck Center for Comparative and Functional Genomics.

We used GeneMapper 3.7 to confirm allelic size variants at each locus for each individual. Mean expected heterozygosities were calculated as an estimate of the total genetic diversity within the lakes for each species. We then calculated pairwise F_{ST} among all lakes to determine the level of among lake differentiation. We used sequential Bonferroni correction (Rice 1989) and exact tests to assess the significance of pairwise F_{ST} values. To test if microsatellite variation reflected patterns of community composition, we performed an AMOVA which partitioned the genetic variance 1) between clusters, 2) among lakes within clusters and 3) within lakes (Excoffier et al. 1992). Analyses were performed in Arlequin v3.1 (Excoffier et al. 2005).

To test the hypothesis that spatial proximity among the lakes influenced patterns of genetic differentiation, we used Mantel tests in IBDWS to compare the pairwise F_{ST} matrices to the geographic distance matrices. We then tested the hypothesis that differences in genetic diversity were related to time since colonization of each species in each lake. To test this hypothesis, we compared mean expected heterozygosity to time since colonization using Pearson's correlation.

Finally, we compared diversity at the trait and neutral genetic levels in two ways. First, we asked whether the amount of quantitative genetic variation was correlated with levels of population genetic variation by comparing the mean heterozygosity to the standard deviation of size at maturity in each population. We then tested whether among

population differentiation in quantitative and population genetic measures were correlated using Q_{st} and F_{ST} matrices.

4.4 RESULTS

4.4.1 Community Structure

Sediment core and water column samples revealed that although seven cladoceran taxa have colonized all eight lakes, current community structure is extremely different (Fig. 4.1; Table 4.1). The core data further suggest that despite species colonizing at different times over the past century, the order or time of arrival did not determine current community structure (Table 4.1). Cluster analysis identified two main groups of lakes with high bootstrap support (Fig. 4.1b). One cluster (C) contained all deep lakes (i.e., those that stratify thermally and have a cold-water summer refuge). Each of these assemblages was dominated by *Daphnia pulicaria* (>80% of individuals). This cluster was separated with high bootstrap support from shallower lakes that either have no summer stratification (cluster A) or stratify with anoxic (or occasionally anoxic) hypolimnia, and therefore, have no summer refuge (cluster B). These lakes were dominated by smaller-bodied species. We found no relationship between community similarity and geographic distance (Mantel test: $r = 0.09$, $p = 0.75$).

4.4.2 Trait Variation

For both *D. dentifera* and *D. pulicaria*, we found evidence that body size distributions were differentiated among lakes (Fig. 4.2a, b). Mean body size of both *D. dentifera* ($F_{5, 295} = 10.43$, $p < 0.001$) and *D. pulicaria* ($F_{4, 216} = 3.59$, $p = 0.007$) differed among the lakes with 15.0% and 5.8% (respectively) of the variation attributable to among lake differences. There was no overall pattern in mean body size between the deep lake cluster and the shallow lake cluster for either species (*D. dentifera*: $F_{1, 4} = 2.26$, $p = 0.21$; *D. pulicaria*: $F_{1, 3} = 1.49$, $p = 0.31$). Additionally, there was no relationship between mean body size and epilimnetic resource richness for either species (*D. dentifera*: $r = 0.054$, $p = 0.92$; *D. pulicaria*: $r = 0.529$, $p = 0.36$). There was some overlap in body size measurements for each species on both the regional and local scales, although the degree of this overlap differed greatly among the lakes (e.g., the body size range of *D. pulicaria*

completely encompassed the range for *D. dentifera* in Deep Lake, while the two ranges did not overlap at all in Long Lake) (Fig. 4.2a, b).

When we assessed the genetic component of size subdivision using clones grown in a common garden, subdivision of size variation among the lakes remained significant (Fig. 4.2c, d; *D. dentifera*: $F_{5, 111} = 5.35$, $p < 0.001$; *D. pulicaria*: $F_{4, 90} = 5.34$, $p < 0.001$), and a greater proportion of the explainable genetic variation was associated with among lake differences (*D. dentifera*: 22.5%; *D. pulicaria* 27.9%). Additionally, body size showed significant broad sense heritabilities for both species (*D. dentifera* 0.54 ± 0.06 SE, *D. pulicaria* 0.40 ± 0.13 SE). We also found that deep lakes had larger size at maturity for *D. pulicaria* ($F_{1, 3} = 10.99$, $p = 0.045$), but there was no pattern for *D. dentifera* ($F_{1, 4} = 0.19$, $p = 0.68$). Average pairwise quantitative trait differentiation (Q_{ST}) for size at maturity in each species was low (bootstrap mean [95% CI]: *D. pulicaria*: 0.128 [0.054, 0.206]; *D. dentifera*: 0.113 [0.050, 0.190]). For *D. pulicaria*, there was some downward bias in the bootstrap estimate relative to the raw data estimate (0.176), though the estimate for *D. dentifera* matched closely (0.114). This bias is partly due to variation among populations in clonal sample size in the *D. pulicaria* dataset. For *D. pulicaria*, much of the among population differentiation was driven by High Lake which had a smaller mean body size and little variance overlap with two of the populations (Fig. 4.2d; pairwise Q_{ST}'s ranged from 0.11 to 0.73). The lower mean Q_{ST} value for *D. dentifera* results from greater among population overlap in trait values (Fig. 4.2c). We did find support for the hypothesis that body size distributions were influenced by spatial isolation of habitats for *D. dentifera* (Mantel test: *D. dentifera* $r = 0.75$, $p = 0.007$), but this was not the case for *D. pulicaria* ($r = -0.29$, $p = 0.771$).

4.4.3 Microsatellite Variation

We found moderate levels of genetic diversity within the Kickapoo lakes for both species of *Daphnia* (Table 4.2). Contrary to the phenotypic results, we found no evidence that microsatellite variation was partitioned among community clusters. However, a considerable portion of the genetic variation was distributed among populations (*D. dentifera*: 25%; *D. pulicaria*: 14%; Table 4.3a). This provides support for neutral divergence of these markers. This among population variation is reflected in moderate to high pairwise F_{ST} estimates for both species (Table 4.3b). Mean F_{ST} values across all

populations were higher in *D. dentifera* (0.21 ± 0.04 SE) than for *D. pulicaria* (0.13 ± 0.02 SE).

Despite the small distances between each of the lakes (max: 2.8 km), we found evidence that spatial dispersal structured population genetic differentiation for *D. pulicaria* (Mantel test: $r = 0.78$, $p < 0.001$). However, this was not the case for *D. dentifera* ($r = 0.31$, $p = 0.13$). We found no evidence for a positive relationship between microsatellite variation (heterozygosity) and time since population founding (Pearson's r: *D. dentifera*: $r = 0.145$, $p = 0.82$; *D. pulicaria*: $r = 0.412$, $p = 0.59$)

We found no evidence for similar effects of dispersal on genetic and phenotypic levels of biological diversity. Variation in size at maturity within populations was not related to underlying molecular heterozygosity (Pearson's r: *D. dentifera* $r = 0.054$, $p > 0.05$, *D. pulicaria* $r = 0.529$, $p > 0.05$), and there was no relationship between quantitative (Q_{ST}) and population genetic (F_{ST}) divergence (Mantel tests: *D. dentifera* $r = 0.26$, $p = 0.204$, *D. pulicaria* $r = 0.06$, $p = 0.507$). Taken together, these results suggest the independent evolution of the neutral genetic from the quantitative genetic and community levels of biodiversity in this system.

4.5 DISCUSSION

While it is apparent that ongoing dispersal was not substantial enough to homogenize species, trait or genetic composition, dispersal within this metacommunity did not result in the same pattern of sorting at each level of biological diversity. Assembly sequences varied among communities, but local interactions have resulted in species sorting along ecological gradients after 50 – 80 years. Despite obvious differentiation among populations, variation at the phenotypic and neutral genetic levels was substantial within populations. Such variation is essential for community assemblages to change in response to environmental variation (Leibold and Norberg 2004, Norberg 2004). Sediment records show that such complex adaptive changes have occurred as our community composition has changed over the past century. Dispersal through space and time continues to allow the maintenance and redistribution of species, trait and genetic variation within the metacommunity.

Our species composition results suggest communities are primarily structured by species sorting processes. Current cladoceran composition of the lakes is similar to that observed in other Midwestern kettle lakes (e.g., Tessier and Welser 1991), and there is no evidence that local community composition is reflective of isolation by distance (dispersal limitation). After only ~60 years, our deep stratified lakes with a hypolimnetic summer refuge are dominated by the largest cladoceran species, *Daphnia pulicaria*, while the more shallow lakes, or those with anoxic hypolimnia, contain a wider array of smaller bodied species (*D. dentifera*, *D. ambigua, D. parvula*, and *Ceriodaphnia*). Much like Tessier and Welser (1991), our intermediate depth lakes are dominated by medium bodied *D. dentifera.* This pattern is especially striking when considering paleolimnological evidence. Sediment cores show each lake has been colonized at some point by all of these species, and the colonization order differed across the region. This suggests both assembly history and dispersal do not limit community composition in this series of lakes. Other aquatic communities also show considerable evidence for species sorting. Permanence gradients have long been known to influence community structure, affecting all levels of the trophic hierarchy (e.g., Tessier and Woodruff 2002). Additionally, variation in disturbance frequency (e.g., permanance: Urban 2004, fish introduction/removal: Howeth and Leibold 2008), dispersal mode (Vanschoenwinkel et al. 2007), and geographic structure (Cottenie et al. 2003) affect the strength of species sorting.

Although species sorting provides the dominant structuring force in these systems, we found low levels of presumably less adapted species in all lakes (e.g., *D. pulicaria* in shallow, unstratified lakes: Cáceres et al., unpublished data). While coexistence is maintained through a number of mechanisms (e.g., genetic diversity, resource and apparent competition, predation-competition tradeoffs, storage effect - Pimentel 1968, Tilman 1982, Warner and Chesson 1985, Chesson 2000, Vellend 2006), dispersal across space or through time probably plays a prominent role in maintaining coexistence (e.g., through source-sink dynamics: Mouquet and Loreau 2003). At the community level, this coexistence allows shifts in species abundance and composition in response to environmental change (Leibold and Norberg 2004). For example, sediment records and water column data show that *D. ambigua* was once much more common in

Sportsmans' Lake but has recently been absent from the water column (Cáceres et al. 2005) Additionally, for lakes most sensitive to environmental fluctuations (intermediate depth lakes with a temporally variable refuge), interannual abundance data show fluctuations in the dominant species over the last five years (Cáceres et al., unpublished data). Dispersal among communities and sediment egg banks likely provide the buffering capacity to allow these rapid evolutionary changes (Hairston and Kearns 2002, Leibold et al. 2004).

We saw mixed support for our hypothesis that body size distributions would group by community cluster. Limnological properties that influence species assemblages also determine the interspecific interactions that shape life history variation. For example, lake depth influences refuge size and fish predation intensity (Tessier and Welser 1991). Interspecific interactions with fish are known to influence mean body size, even within a single season, and smaller body size is expected where fish predation is intense (Tessier et al. 1992). Accordingly, we observed larger size at maturity for *D. pulicaria* in the deep lakes. However, geographic distance, rather than habitat characteristics, appeared to influence these trait patterns in *D. dentifera*. This suggests dispersal is not high enough to homogenize *D. dentifera* trait values across the system of lakes and some sorting and evolution of traits is occurring, but it is not strong enough to overcome spatial structure. Moreover, a substantial proportion of the overall genetic variance resided within communities. While indeterminate growth, multiple age classes of *Daphnia*, and transient ecological differences contribute to this phenotypic variation in the field, nearly three quarters of the quantitative genetic variance was within lakes (among clones) in the laboratory study. This suggests the maintenance of substantial variation within populations despite the potential for strong selection. The maintenance of such genetic variation through time may provide a buffer against interannual disturbance and contributes to the adaptive capacity of the system (Leibold and Norberg 2004, Norberg 2004).

Despite being relatively young populations, microsatellite variation was moderate for both species of *Daphnia.* Additionally, genetic differentiation was substantial among communities considering their proximity and shared history (compare to mean $F_{ST} = 0.39$ for *D. pulicaria* - Morgan et al. 2001). That the genetic differentiation among populations

was not grouped by community clusters supports the contention that microsatellite loci are diverging neutrally. However, the differing patterns of spatial autocorrelation for the two species suggest history and dispersal continue to shape neutral genetic differentiation. There are a number of possibilities for these patterns. First, differences in the inherent dispersal ability of the two species may influence genetic diversity, spatial autocorrelation and pairwise differentiation. For example, species-specific differences in ephippial buoyancy (Cáceres et al. 2007, Slusarczyk and Pietrzak 2008) may contribute to different emigration rates. Alternatively, population age may influence genetic diversity and divergence (Haag et al. 2005, Louette et al. 2007). While we observed no effect of time since colonization on the genetic diversity *within* species, in every case, whichever species colonized the lake first had higher genetic diversity. Since five of the six lakes were most recently colonized by *D. pulicaria* (despite both species being present in the lake complex for decades), a lower relative dispersal rate may explain the observation of lower genetic diversity and genetic isolation by distance in this species.

It is apparent from our study that dispersal influences species, trait and genetic composition, but the consequences of dispersal differ across levels of biological diversity. Habitat characteristics such as lake depth and its influence on higher trophic levels directly influence optimal size distributions in aquatic zooplankton communities (Tessier and Welser 1991). Such interactions affect both community structure and body size within species. Since microsatellite variation is purportedly neutral, there is not a predictable direction for shifts in the distribution of the variance. Variance shifts may simply result from drift and gene flow (or clonal selection for asexual species), and since the selective agent operating at the trait or community level is not directly influencing neutral genetic variation, no pattern is observed. Similar results have been observed between mean genetic diversity and phenotypic variation or heritability (Morgan et al. 2001). Thus, across a metacommunity, ecological partitioning may occur at multiple levels, but with different underlying processes driving changes. However, that is not to say that variation at one biodiversity level does not influence other levels. Genetically diverse populations have been shown to resist the invasion of new species despite ongoing dispersal (De Meester et al. 2007). Similarly, local adaptation may reduce the

success of invading genotypes or species into established communities (Shurin 2000; De Meester et al. 2002).

Comparisons of Q_{ST} and F_{ST} have become a common test to understand evolutionary mechanisms underlying quantitative trait differentiation among populations (e.g., Spitze 1993, Merila and Crnokrak 2001, Le Corre 2005, Porcher et al. 2006). While there are criticisms of the methods (e.g., O'Hara and Merila 2005), the Q_{ST} - F_{ST} comparison assumes neutral markers diverge only because of mutation, drift and gene flow. Thus, when Q_{ST} is greater than $F_{ST,}$ another force (i.e. divergent selection) must be the cause of population differentiation (Spitze 1993). Additionally, when populations are isolated from one another reduced gene flow will not only allow neutral divergence of populations, but also local and independent adaptation of quantitative traits. Thus, where populations diverge independently, a positive correlation between Q_{ST} and F_{ST} is hypothesized and has been observed (Merila and Crnokrak 2001). Studies on *Daphnia* have clearly demonstrated such patterns of quantitative differentiation and suggested local adaptation may be a cause of such differentiation (e.g., Morgan et al. 2001). In our system, however, Q_{ST} was low and not different from F_{ST}. This may suggest gene flow is high, or that populations have been founded from the same gene pool (shared founder effects). Either way, examining the system at three levels of biodiversity allows us to detect the effects of selection in these young habitats of similar origin, even if total among population differentiation is lower. Understanding how dispersal interacts with history and habitat to influence biodiversity allows us to better explain levels of intrapopulation variation in species composition, life history traits and genetic variation.

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4.8 TABLES

Table 4.1

Colonization year of cladoceran taxa recoverable from sediment cores. *Daphnia ambigua* and *D. parvula* ephippia were not distinguishable from one another and thus are grouped together.

Lake	***Ceriodaphnia reticulata***	***Daphnia ambigua - parvula***	***Daphnia dentifera***	***Daphnia pulicaria***
#6	1967	1928	1979	1949
Clear	1928	1926	1987	1996
Deep	1969	1980	1985	1971
Emerald	1964	1926	1963	1959
High	1950	1939	1967	1998
Inland	1927	1927	1927	2004
Long*	1	1	3	4
Sports	1954	1954	1963	1966

*A cliff was pushed into Long Lake disrupting the assumption of equal sedimentation rate over time. Therefore, the colonization sequence is provided.

Table 4.2

Mean expected heterozygosity (± SD) of *Daphnia dentifera* and *D. pulicaria* in Kickapoo lakes

Lake	***D. dentifera***	***D. pulicaria***
Clear	0.517 ± 0.189	0.421 ± 0.245
Deep	0.272 ± 0.242	0.374 ± 0.258
High	0.373 ± 0.259	0.274 ± 0.342
Inland	0.427 ± 0.186	-
Long	0.577 ± 0.108	0.406 ± 0.292
Sports	0.468 ± 0.252	0.398 ± 0.238
Total	0.544 ± 0.081	0.432 ± 0.233

Table 4.3

Results of a) AMOVA and b) pairwise F_{ST} for microsatellite differentiation among populations of *Daphnia dentifera* and *D. pulicaria* in Kickapoo lakes. Lakes were grouped by their community cluster (Fig. 4.1b) for the AMOVA model. F_{ST} values are below the diagonal, while exact test significance values are above the diagonal (+ means $p < 0.05$). Boldface F_{ST} values are significantly greater than zero after sequential Bonferroni correction ($p < 0.05$). Both AMOVA and F_{ST} results were calculated in Arlequin v3.1 for the all genotyped individuals.

a) AMOVA Tables

Daphnia dentifera

Variation Source	**d.f.**	**Sum of Squares**	**Variance Components**	**% variation**
Among clusters	1	8.3	-0.015	-1.33
Among lakes				
within clusters	4	35.6	0.279	25.39
Within lakes	172	143.6	0.835	75.94
Total	177		187.5	1.100

Daphnia pulicaria

Variation Source	**d.f.**	**Sum of Squares**	**Variance Components**	**% variation**
Among clusters	1	5.6	-0.015	-1.41
Among lakes				
within clusters	3	19.2	0.150	13.86
Within lakes	183	173.8	0.950	87.54
Total	187	198.6	1.085	

Table 4.3 (cont.)

b) Pairwise F_{ST} values

Daphnia dentifera

	Clear	Deep	High	Inland	Long	Sports
Clear		+	+	+	-	+
Deep	0.093		+	+	+	+
High	**0.159**	**0.309**		+	+	+
Inland	**0.310**	**0.539**	**0.336**		+	+
Long	0.055	**0.258**	0.092	0.132		+
Sports	**0.196**	**0.406**	**0.144**	**0.147**	0.023	

Daphnia pulicaria

	Clear	Deep	High	Long	Sports
Clear		+	+	+	+
Deep	**0.170**		+	+	+
High	**0.172**	**0.130**		+	+
Long	**0.104**	-0.001	**0.104**		+
Sports	**0.079**	**0.214**	**0.180**	**0.156**	

4.9 FIGURES

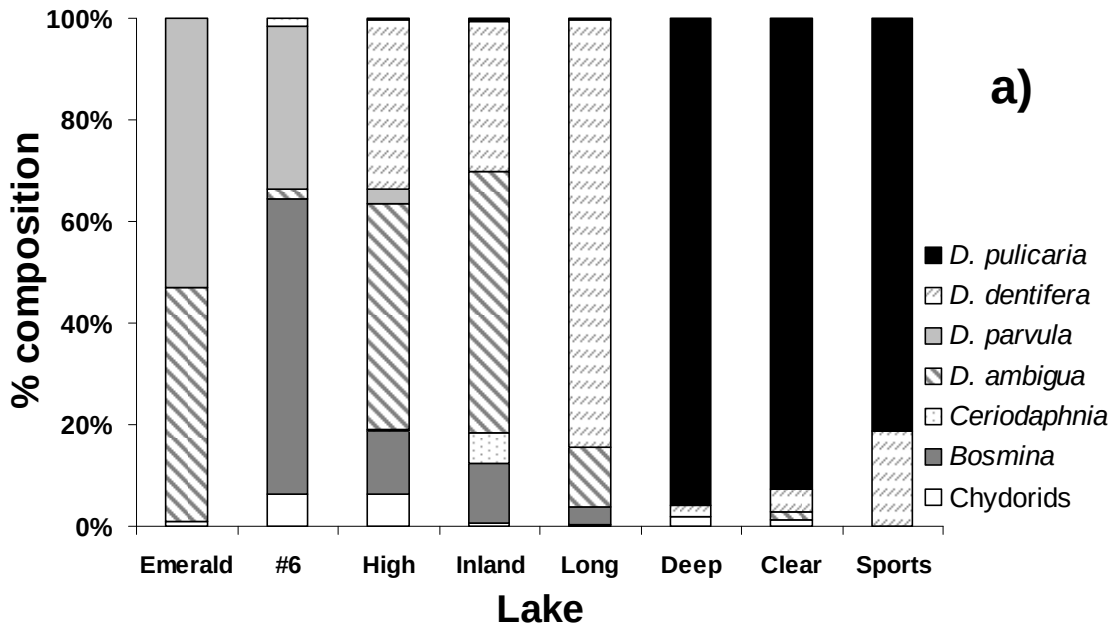

Figure 4.1

Cladoceran composition and structure in eight lakes at Kickapoo State Park. a) Percent composition of each species. Lakes are ordered from shallowest to deepest (left to right) and taxa are ordered from smallest to largest (bottom to top). b) Cluster dendrogram of cladoceran community similarity. We used agglomerative clustering with the Bray-Curtis dissimilarity index and complete linkage method on the square root transformed cladoceran count data. Bootstrap values (1000 randomizations) are above each node. Lake groups are labeled by depth: A) shallow, unstratified lakes, b) intermediate depth, stratified, late-summer anoxic lakes, and C) deep, stratified refuge lakes.

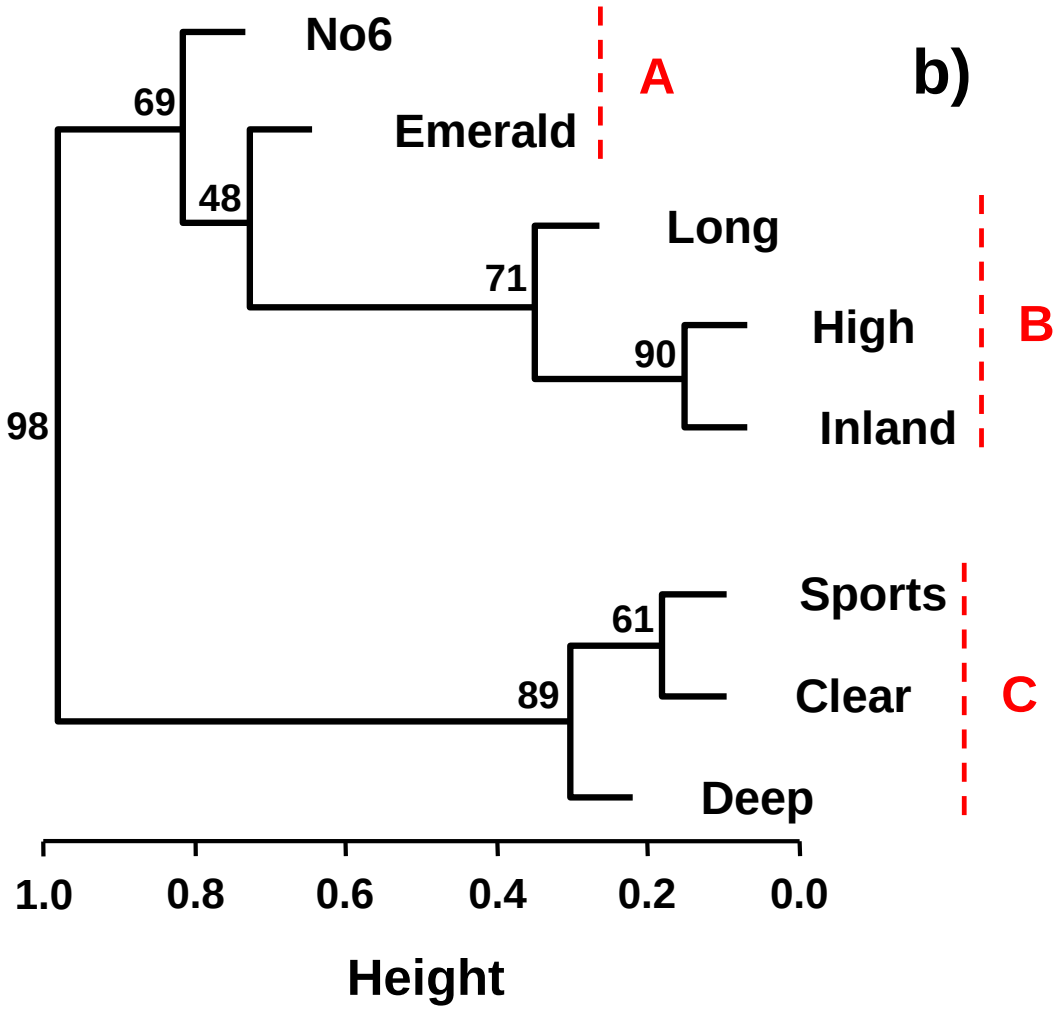

Figure 4.1 (cont.)

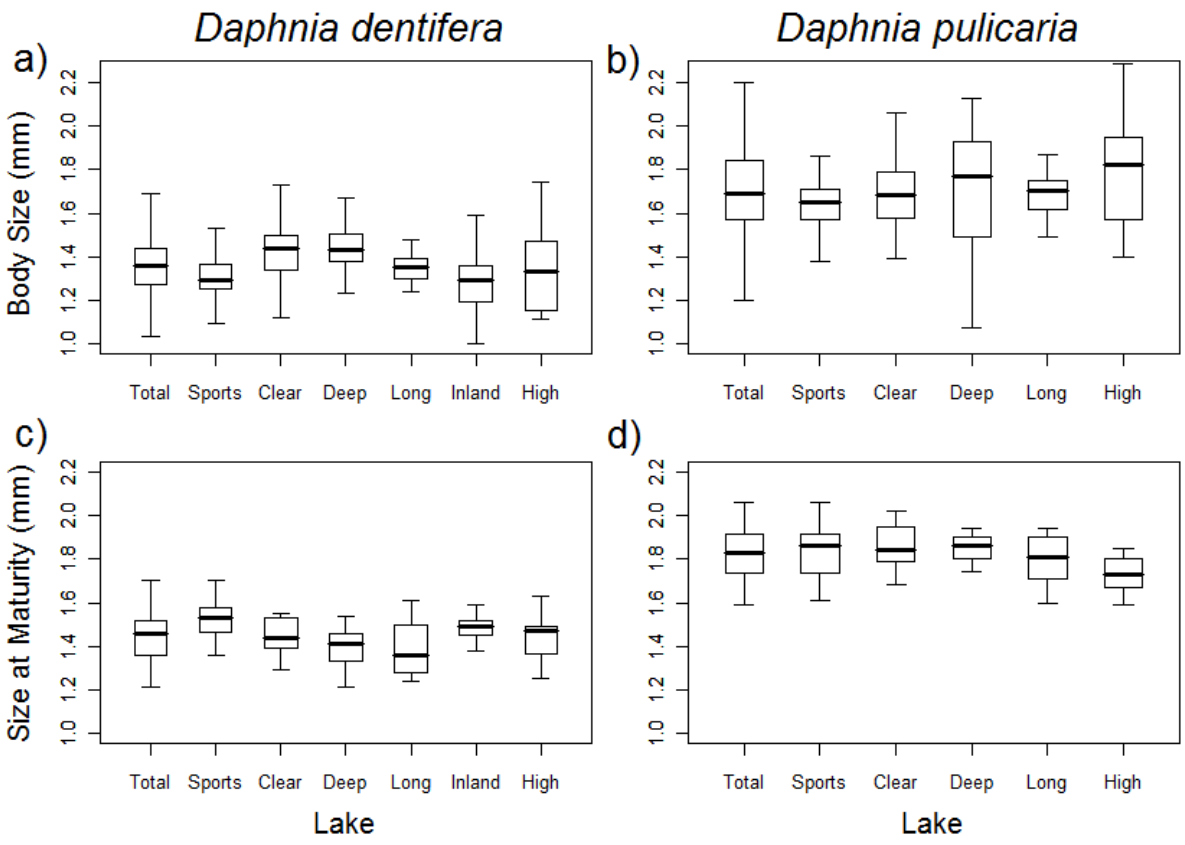

Figure 4.2

Trait distributions of *Daphnia dentifera* and *D. pulicaria* for body size in the field (a,c) and size at maturity (b,d) for six Kickapoo lakes. Boxes represent the interquartile range and whiskers extend to outliers within 1.5 IQR of the box. The leftmost boxes encompass the total range of variation for each species across the five lake region, and lakes decrease in depth from left to right. Too few *D. pulicaria* were recovered from Inland Sea for either experiment.

Appendix A: A test for cladoceran reproduction within traps

To examine whether zooplankton were reproducing within the traps, I measured dispersal to 10 m traps for 3, 6 and 9 days between May 4 and 13 – the experimental run experiencing the highest density of cladocerans in the pond. Three buckets were placed next to each other on each transect and one of the buckets was emptied on each sampling day following the procedures outlined in the methods section. I then compared propagule accumulation over time of potentially reproducing cladocerans and non-reproducing cladoceran ephippia with ANCOVA. Equal slopes of these regression lines would imply that the rate of accumulation was the same for both the cladocerans and their ephippia, suggesting reproduction was not occurring.

I found no significant difference in the rate of accumulation of ephippia versus cladocerans over the 9 day trial, as evidenced by the equal slopes of the regression lines (Fig. A.1; ANCOVA $F_{1,44} = 0.38$, $p = 0.54$). Additionally, I observed adult cladocera in buckets sampled after each interval, while only nine juvenile cladocera in one trap were observed from all of the experiments (216 traps; and juveniles were excluded from all count analyses). This provides the strongest evidence for no reproduction and for the transport of adults (as opposed to hatching of individuals from ephippia). These results suggest that reproduction of cladocerans within traps was not a major concern.

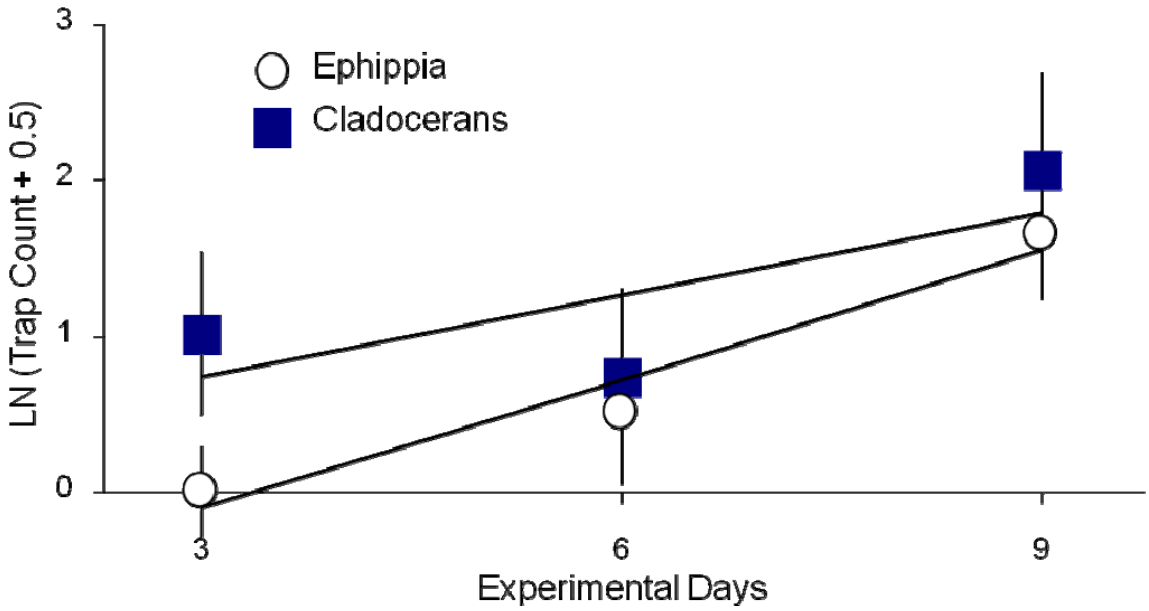

Figure A.1

Analysis of reproduction occurring within traps. The natural log number of ephippia within traps over the course of the nine-day experiment is compared to the natural log number of cladocerans within the traps. That there is no difference between the slopes of these lines (ANCOVA $F_{1,44} = 0.38$, $p = 0.54$) suggests cladocerans are not increasing in the traps at a faster rate than the ephippia. This provides support for the assumption cladocerans are not reproducing in the traps during nine days.

Appendix B: Detailed model assumptions and design

To fit dispersal models to the data, I employed a number of assumptions about data collection, field conditions and models to be fit. First, I assumed the trap data were time-integrated (Turchin 1998). Time-integrated methods are appropriate for instances when organisms are continuously released as earlier dispersers are counted along with late dispersers (appropriate for a continuously releasing pond). Second, I considered the pond a constant source of propagules over each nine-day capture period for my analyses. This average pond density seemed appropriate, as abundances did not change a great deal during any given replicate. Third, I assumed the data followed a point release model. Generally, with a surface of equal density such as a pond, area release methods should be employed. However, area release methods only apply if dispersal is equally probable across the entire surface. When considering pond dispersal, this may be the case for wind and rain as vectors; however, animal vectors primarily use the edges of the pond. As the field data suggested that animals were the primary dispersal vector, the simpler point release model seemed appropriate.

Next, I tested the assumption of no drift or directionality in the field data. I followed the method of Turchin and Thoeny (1993). Since the pond surface was an oblong ellipse, I first found the absolute center of the dispersal array by averaging all of the x and y coordinates of the trap locations (x and y calculated from UTM coordinates in GIS). Then, the center of the two dimensional array, weighted for dispersal counts, was calculated for each replicate using:

$$X_j = \frac{\sum_{i=1}^{n} x_i C_{ij}}{\sum_{i=1}^{n} C_{ij}}, \qquad \text{(equation B.1)}$$

where X_j is the weighted mean x coordinate of replicate j, x_i is the coordinate for trap i and C_{ij} is the trap count of trap i in replicate j (Turchin and Thoeny 1993). I tested for significant displacement of the weighted versus absolute center of the array in both the x and y directions using separate t-tests. The weighted center of dispersal (mean ± 1 SE) was displaced 8 ± 5 m (t = 1.58, df = 4, p = 0.19) and -6 ± 6 m (t = -1.12, df = 4, p = 0.33) in the x and y axes, respectively. The magnitude of this mean deviation is relatively

small, as the displaced center remained within the pond limits. Thus, the assumption of no directional bias in dispersal held true at the field site.

I chose four frequently employed empirical models with different behaviors at the head, tail and center of the dispersal kernel to fit the dispersal data (Table B.1). The model head describes the density close to the source, while the tail describes the density far from the source. The inverse power (IP) and negative exponential (NE) models have been used for years. The IP model is the most leptokurtic of the four models, while the NE model is the most platykurtic. The mechanistic negative exponential model (MNE), proposed by Turchin and Thoeny (1993), is a variant of the typical negative exponential model that is derived from diffusion principles. The Students' two-dimensional t model (2Dt) was developed by Clark et al. (1999) as a mixture of convex head and fat tailed models. Each of these models assumes a constant source of propagules among replicates. As the pond density changed between sampling events, I included it as a covariate in the model formula to control for initial dispersal source size. The NE, MNE and IP models were fit to the data by maximum likelihood estimation of generalized linear models assuming a Poisson error structure and a log link function. The log pond density (D) was treated as an offset variable for the NE and IP models. For the MNE model, log(D) minus ½ the radial distance (r) served as the offset variable. The models were fit using the 'glm' procedure in R 2.3.1 (R Development Core Team 2006). The 2Dt model was fit via maximum likelihood estimation using an algorithm that minimized the negative log-likelihood of the model ('optim' in R). Again, a Poisson error structure was assumed. To choose among models, I calculated the AIC and ΔAIC values for each of the models and selected those models with the lowest AIC and $\Delta AIC < 2$ (Burnham and Anderson 2002).

Using the best model and its parameters, I derived a number of statistics to describe dispersal at this spatial scale, including the distance to which a fraction of the dispersers traveled, the number of pond organisms dispersing on a daily basis and the percent of pond organisms dispersing daily. For the MNE model using typical point source dispersal, Turchin and Thoeny (1993, eqn. 9) describe an integral equation to approximate the median dispersal distance, or the area encompassing 50% of the dispersers. However, in this case, while a point source equation provided an appropriate fit to the data, it does not correctly account for the area dilution effect when radiating

from a pond of a given area. As such, a modified probability density function (PDF) based on the fitted parameters was necessary to adequately model radiating distance. The most mathematically tractable form of this function is a discrete function. Simply stated, this function is the total number of dispersers reaching the 1 meter wide annulus (i.e., the area between two concentric circles) at a given distance from the pond divided by the total number of dispersers over the entire dispersal surface, or

$$\text{PDF} = \frac{F(r)\,A(r)}{\sum_{x=0}^{\infty} F(r)\,A(r)}, \qquad \text{(equation B.2)}$$

where *F(r)* is the average predicted density in the annulus at distance *r* and *A(r)* is the area of the 1 m wide annulus. Based on the NE model,

$$F(r) = \frac{D}{\pi\ 0.15^2} A\left(\frac{Exp[\frac{-r}{B}] + Exp[\frac{-(r+1)}{B}]}{2}\right). \qquad \text{(equation B.3)}$$

(This format for *F(r)* can be applied to any fit model.) To calculate the area surrounding the pond, *A(r),* I measured the area from the pond edge to each of the five distance classes using tools in ArcGIS. I then fit a quadratic regression to find area over distance. For this study,

$$A(r) = 3.1118r^2 + 84.108r - 32.086 \quad (R^2 = 1). \qquad \text{(equation B.4)}$$

To calculate the median dispersal distance, equation B.2 was set to 0.5 and solved for $r_{0.5}$:

$$0.5 = \frac{\sum_{x=0}^{r\,0.5} F(r)\,A(r)}{\sum_{x=0}^{\infty} F(r)\,A(r)}. \qquad \text{(equation B.5)}$$

However, this equation is not a closed function in this discrete form. As such, I ran it iteratively for all potential values of r_x and chose the value for *r* closest to 50%. Finally, to calculate the percent of individuals leaving the pond daily, I set pond count (*D*) to 1 and solved for the denominator of equation B.2. All calculations were performed in Mathematica 9.2 (Wolfram Research, Champaign, IL).

Table B.1

Models fit to the cladoceran dispersal data. *A* and *B* are fitted parameters, *D* represents the count of individuals within the pond during a given replicate, and *r* is the distance from the pond.

Model	**Dispersal kernel *C(r)***
Mechanistic Negative Exponential (MNE)[a]	$D\,A\,r^{-1/2}\,\mathrm{Exp}[-r/B]$
Negative Exponential (NE)	$D\,A\,\mathrm{Exp}[-r/B]$
Inverse Power (IP)	$D\,A\,r^{-B}$
Student's two dimensional t (2Dt)[b]	$\frac{D\,A}{\pi\,B[1+\frac{r^2}{B}]^{A+1}}$

[a]Turchin and Thoeny (1993); Turchin (1998) [b]Clark et al (1999)

Appendix C: A list of zooplankton taxa in Center Pond

Cladocera

*Daphnia obtusa**

*Simocephalus vetulus**

Chydorids*

*Scapholeberis mucronata**

*Ceriodaphnia reticulata**

*Bosmina longirostris**

Copepods

Calanoida*

Cyclopoida*

Harpactacoida*

Rotifers

Bdelloidia*

*Lecane**

*Brachionus**

*Platyias**

*Cephalodella**

*Keratella**

*Polyarthra**

Trichocera

Kellicottia

*Those taxa marked with an asterisk were recovered in traps.

Appendix D: Zooplankton densities in Center Pond and bucket traps

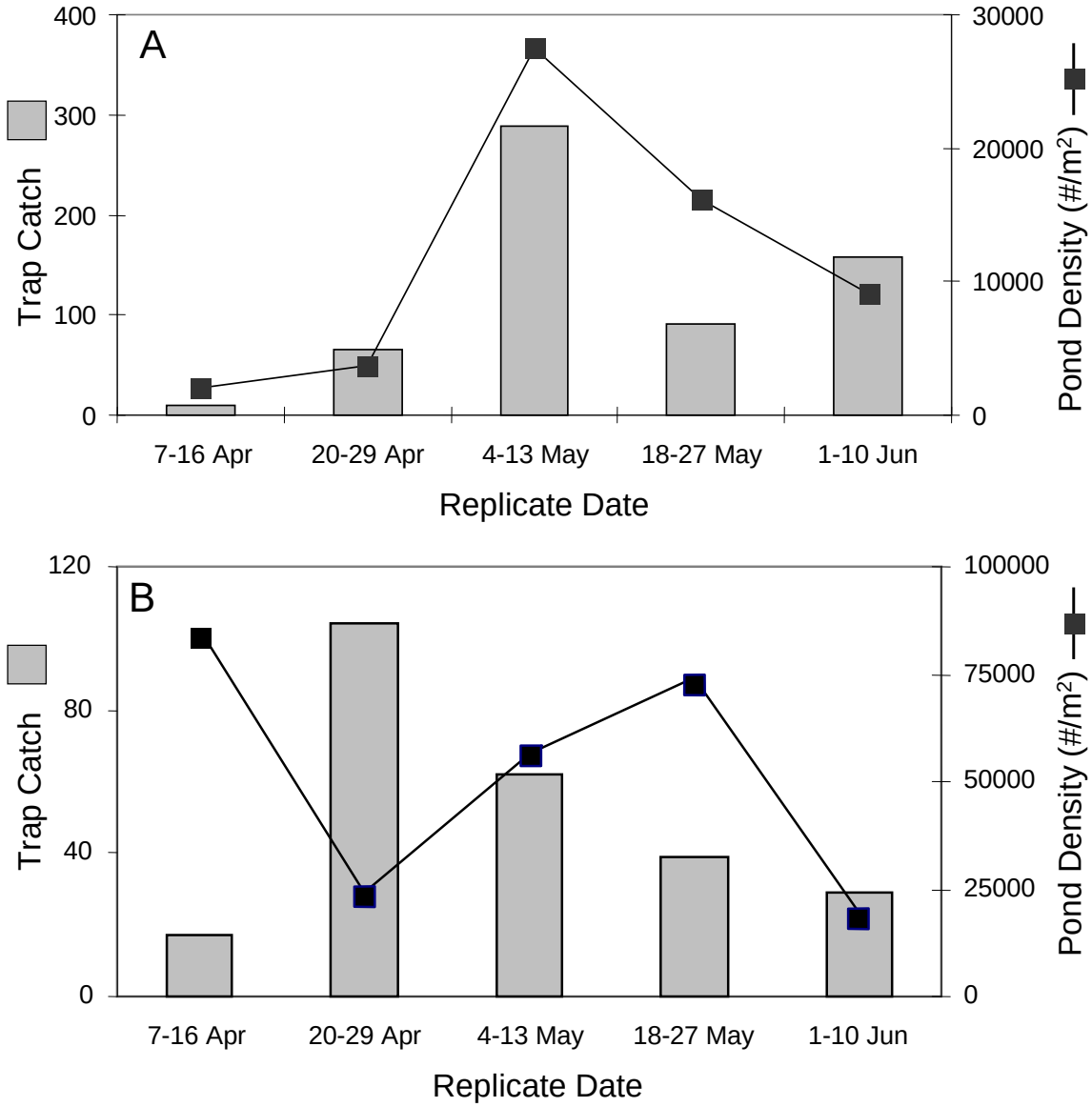

Figure D.1

Trap catch relative to pond density for A) cladocerans and B) copepods. Trap catch is the sum of animals collected from all buckets during one replicate. Five experimental replicates ran between 7 April 2004 and 10 June 2004. Replicate 3 captured the peak abundance of cladocerans in the pond of the entire growing season. The cladoceran trap catch closely follows pond density while, the copepod figure suggests trap capture poorly tracked pond density.

Appendix E: Estimation of the lower bound of attractiveness for the dispersal model

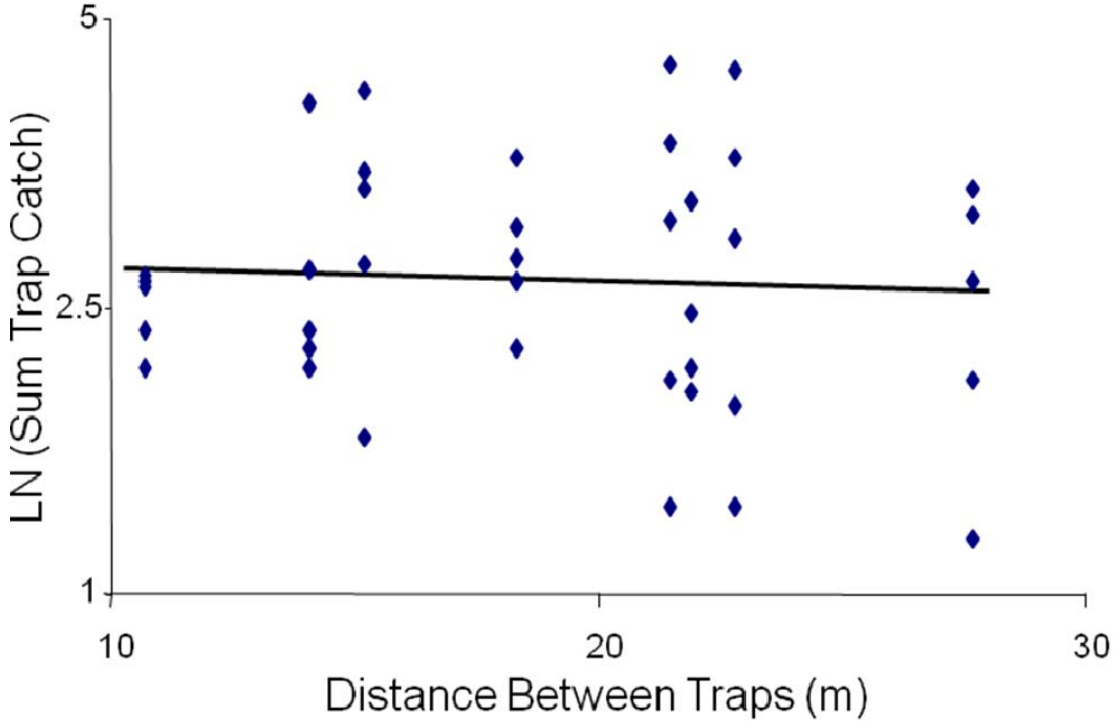

Figure E.1

The sum trap catch is the total number of individuals caught in adjoining traps during a given replicate. This sum is regressed against the distance between those two traps. As there is no relationship between the number of individuals trapped and the distance between neighboring traps (at the 10 m distance; $F_{1,38} = 0.14$, $P = 0.71$), the collecting areas of the traps are not assumed to overlap. Thus, one half the distance between the closest traps was used as a conservative estimate for collection area.

Appendix F: Locations and characteristics of hatching fraction populations

Table F.1

Field locations and characteristics of populations included in the 2005 and 2006 hatching fraction studies. Variables:Light_tray – percent of ambient light reaching the hatching tray; Light_atten – the light attenuation constant (Wetzel 2001); Tray_d – depth of the hatching tray in meters; Max_d – maximum depth in meters at high water (>1.2 m values are not included in statistical analyses); DO_pct and DO_mg – percent and milligrams of dissolved oxygen measured by DO probe; Cond – conductivity in µS/cm; Chla - µg/L chlorophyll in water column; TP – total phosphorus in µg/L; Neighbors – number of ponds within 1 km during spring; Egg-filling – percent of ephippial chambers containing eggs during initial collection. Dots indicate missing data.

Pond Name	Pond ID	Longitude (N)	Latitude (W)	State	Light_tray
3 Rivers 2	3r2	41.8484	-85.75025	MI	0.2191
Bridge South	BridgeS	40.12212	-87.73674	IL	0.3763
Busey	Busey	40.12868	-88.2131	IL	0.3055
Center	Center	40.13291	-88.14004	IL	0.85
Duffy Road 1b	DR1b	42.6026	-85.47924	MI	0.8282
Duffy Road 2a	DR2a	42.60242	-85.47997	MI	0.4094
Duffy Road 4	DR4	42.60443	-85.48932	MI	0.2465
Engle	Engle	42.71543	-85.36863	MI	0.135
Erway 5b	Er5b	42.61503	-85.39925	MI	0.0179
Campground	Camp	42.32619	-85.33459	MI	0.3704
Fulton	Ful	42.10064	-85.31939	MI	0.2854
Baby	Baby	42.58478	-85.41745	MI	0.7895
Potato Creek 2	PC2	41.5402	-86.35744	IN	0.4583
Rainbow	Rain	42.61642	-85.47557	MI	0.1923
Robertson6	Rob6	42.74553	-85.42217	MI	0.4231
Mallard	RWF2	41.71519	-89.18073	IL	0.6875
Pothole	RWF5	41.70474	-89.19486	IL	0.3407
POVI	POVI	42.71878	-85.38793	MI	0.3407
Top	Top	40.24203	-87.78242	IL	0.6552
West Gull	WG	42.41289	-85.43919	MI	0.0783
Willow Slough C	WSC	40.96626	-87.52456	IN	0.2303
Wildwood	WW	42.58858	-85.48839	MI	0.786

Table F.1 (cont.)

Pond ID	Light_atten	Tray_d	Max_d	DO_Pct	DO_MG	pH
3r2	0.5078	0.57	0.6	79.2	9.3	7.1
BridgeS	0.5841	0.63	1.15	51.5	5.59	7.6
Busey	0.6295	0.4	.	92.7	10.02	7.9
Center	0.3529	0.2	1.2	80.8	8.1	7.5
DR1b	0.1594	0.37	0.4	49.4	5.74	6.9
DR2a	0.5478	0.5	0.73	50.1	6.27	8.2
DR4	0.7298	0.56	>1.2	77.2	9.6	8.4
Engle	0.8412	0.49	>1.2	37.9	4.62	6.8
Er5b	.	.	.	76.9	9.69	6.9
Camp	0.6289	0.28	.	43.1	5.6	7.6
Ful	0.6509	0.25	0.45	55	7.24	7.4
Baby	0.3667	0.28	0.45	64.7	8.44	7.2
PC2	0.4402	0.4	0.47	87.2	9.81	6.9
Rain	1.4964	0.43	0.52	47.6	5.79	9.1
Rob6	0.8624	0.25	>1.2	48.8	5.95	7.2
RWF2	0.3698	0.44	0.95	97.6	9.74	9.6
RWF5	0.6681	0.7	1.2	76.2	7.36	7.6
POVI	0.7044	0.25	0.45	60.6	7.53	7.1
Top	0.4833	0.38	0.41	57.4	5.75	7.6
WG	1.4493	0.69	0.9	72.1	9.96	8.3
WSC	0.8656	0.4	0.65	89.3	10.38	7.1
WW	0.3732	0.2	0.52	82.4	10.91	7.5

Table F.1 (cont.)

Pond ID	Cond	Chla	TP	Neighbors	Egg-filling
3r2	108	2.946	81.6	8	100
BridgeS	887	2.447	.	8	94
Busey	1005	6.002	126.6	3	86
Center	545	0.732	103.8	1	93
DR1b	337	1.329	27	16	90
DR2a	510	0.323	278.8	16	100
DR4	597	5.827	62.1	12	88
Engle	68	20.43	158.2	11	93
Er5b	44.7	10.289	129.7	21	96
Camp	334	3.878	295.1	7	92
Ful	260	1.891	325.2	5	86
Baby	348	0.418	43.7	7	100
PC2	92.4	1.074	64.7	7	100
Rain	48	4.492	229.7	8	98
Rob6	268	1.582	41.1	14	92
RWF2	205	1.603	86.7	8	98
RWF5	12.7	5.829	271.4	7	92
POVI	175	0.624	119.1	30	100
Top	360	1.242	56.2	6	94
WG	129	7.769	138.8	3	100
WSC	154	1.007	66.1	3	89
WW	85	5.509	72.5	7	100

Appendix G: Hatching and survival of dormant eggs in 2005

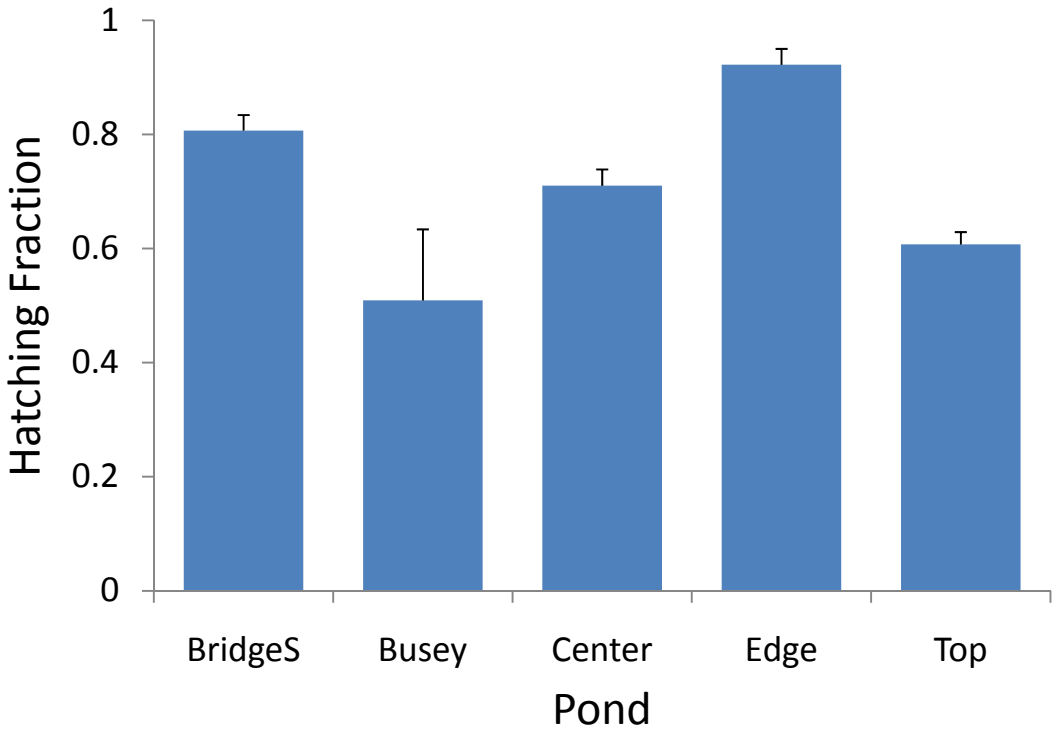

Figure G.1

Mean (± SE) a) hatching fraction and b) dormant egg survival rate of field collected eggs incubated in their own pond during 2005-2006. Hatching rates varied significantly among the ponds, but survival rates did not.

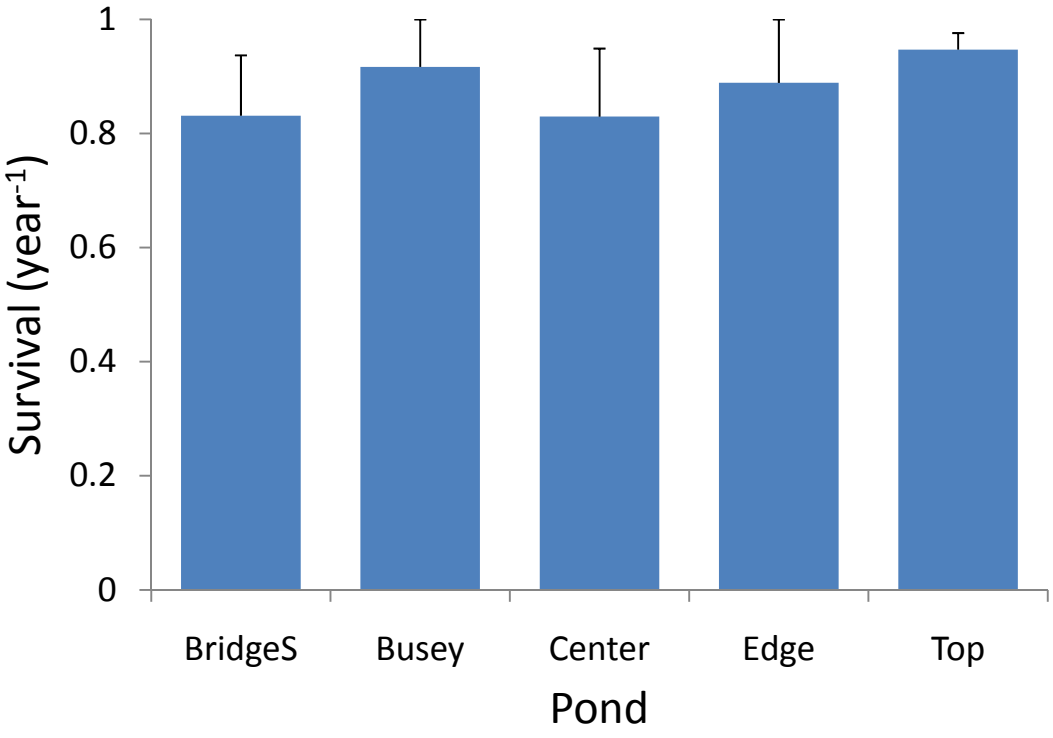

Figure G.1 (cont.)

Appendix H: Design matrix of local adaptation experiment

Each cell contains the number of clones for the particular water by population source interaction. Note that clones were nested within populations for the statistical analysis. Cell numbers are less than the original eight intended for the experiment primarily because clonal mothers did not produce enough neonates during the experimental setup to be included in the experiment. While some individual *Daphnia* died during the course of the experiment, other individuals of the same clone nested within cell (population x resource) completed the experiment permitting an estimates for each replicates. Only two replicates were lost due to experimental deaths (one from Busey (Pop.) x Top (Res.) and one from Dump x Dump).

Population

Resource	BridgeS	Busey	Dump	Top
BridgeS	7	6	5	5
Busey	7	7	4	5
Dump	7	7	4	5
Top	6	6	4	5

Appendix I: Map of Kickapoo State Park

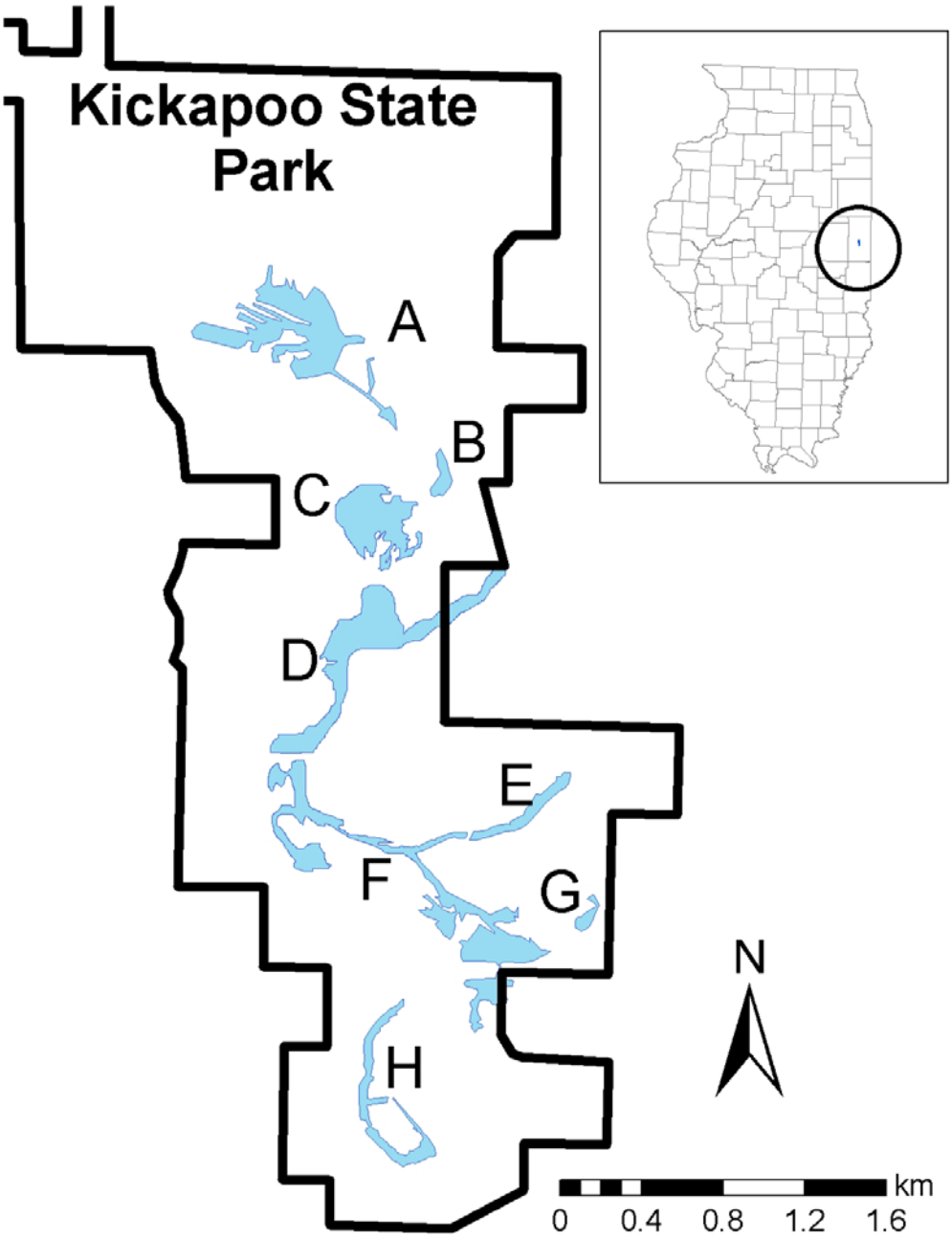

Figure I.1

Kickapoo State Park (40.14°N, 87.74°W) is located in east central Illinois, USA (inset). The park hosts a number of lakes recently created from abandoned strip mines. Eight of these lakes are included in this study: a) Sportsmans' Lake, b) Emerald Pond, c) Inland Sea, d) Clear Pond, e) High Pond, f) Long Lake, g) Deep Pond, and h) Number 6 Pond.

Appendix J: Characteristics of Kickapoo State Park lakes

Table J.1

Characteristics of study lakes located at Kickapoo State Park. Lakes are ordered from shallowest to deepest. Resource richness is the mean juvenile growth rate of a standard *Daphnia pulex-pulicaria* clone grown on epilimnetic water from each lake.

Lake	Origin Date	Surface Area (ha)	Max Depth (m)	Resource Richness
Emerald	1926	1.3	4	0.09
#6	1927	4.2	5	0.65
High	1926	3.5	7	0.15
Inland	1926	7.9	9	0.11
Long	1927	20.7	10	0.23
Deep	1959	0.8	10	0.09
Clear	1926	14.6	16	0.07
Sports	1953	14.4	16	-0.04

Appendix K: Curriculum Vitae

Michael R. Allen

mra142@gmail.com

EDUCATION

PhD in Ecology, Evolution and Conservation Biology, GPA 3.96 — 2009
The University of Illinois at Urbana-Champaign

BS in Biology (Ecology), GPA 3.87 — 2003
Distinction and with Honors in Biology,
The Pennsylvania State University, University Park

RESEARCH EXPERIENCE

Dissertation Research (NSF Fellow, Graduate Research Assistant) — 2003-2009
University of Illinois at Urbana-Champaign (UIUC). Advisor: Dr. Carla Cáceres

Honors Undergraduate Researcher — 2001-2003
Pennsylvania State University - University Park. Advisor: Dr. Katriona Shea

WORK EXPERIENCE

Research Assistant — 2003
Pennsylvania State University - University Park. Advisor: Dr. Ottar Bjørnstad

Research Technician — 2002
Walter Reed Army Institute of Research. Supervisor: Dr. Sheetij Dutta

Interpretive/ Visitors Service Intern — 2001
Monomoy National Wildlife Refuge**.** Supervisor: Debbie Long

PUBLICATIONS

Allen, M. 2007. Measuring and modeling dispersal of adult zooplankton. *Oecologia* **153:** 135-143.

Allen, M. & K. Shea. 2006. Spatial segregation of congeneric invaders in central Pennsylvania, USA. *Biological Invasions* **8**: 509-521.

Allen, M. 2003. Weed invasions in Pennsylvania: a distributional study of two *Carduus* thistle species. Honors Thesis. The Pennsylvania State University.

FELLOWSHIPS AND AWARDS

National Science Foundation Graduate Research Fellowship	2004-2009
Emerging Leader Award. Presented by the Office for LGBT Resources	2008
Robert P. Larsen Award. For service and commitment to campus community	2008
List of Teachers Ranked Excellent by their Students	2008
PEEC Departmental Research Assistantship	2004, 2008
Best Talk by a Junior Student. GEEB Symposium	2006
Graduate Teaching Certificate. Center for Teaching Excellence.	2005
Enhancing Linkages between Mathematics and Ecology Scholarship	2004, 2005
Society of Distinguished Alumni Scholarship	2003
Millman Scholarship in Science	2001-2002
Amos William and Annie Unger Memorial Scholarship	2001-2002
Academic Excellence Scholarship	1999-2003
Eagle Scout	1997

GRANTS

Clark Research Support Grant 2008
Graduate College Travel Support Grant 2008
Program in Ecol., Evol. and Cons. Biology Travel Grant 2004-2006, 2008
Program in Eco., Evol. and Cons. Biology Summer Research Grant 2004-2007
Illinois Wildlife Preservation Fund Research Grant 2005
Schreyer Honors College Summer Research Scholarship 2002
Eberly College of Science Undergraduate Research Grant 2001

TEACHING EXPERIENCE

Teaching Assistant
IB 449: Limnology. (35 students) Prof: Dr. Carla Cáceres 2007
IB 150: Organismal and Evol. Biology. (80 students) Prof: Tracey Hickox 2005
IB 449: Limnology. (30 students) Prof: Dr. Carla Cáceres 2004

Mentoring of Undergraduate Students
Ben Sandkam (2008), John Lofky (2008), Christine Knight (2005-2006), Katy Rende (2004-2005), Dominic Philpott (2004)

SELECTED PRESENTATIONS (6 of 12 total)

Allen, M. Ecological Society of America Annual Meeting. Milwaukee, WI 2008
Does spatial isolation influence the average fitness of temporary pond *Daphnia* populations?

Allen, M., J. Smith, R. Thum, C. Cáceres. ASLO Annual Meeting. St. Johns, NF 2008
Ecological sorting of life history variation in recently formed lakes.

Allen, M. ASLO Aquatic Sciences Meeting. Santa Fe, NM 2007
Genetic and environmental factors control hatching in temporary pond *Daphnia.*

Allen, M. ESA Annual Meeting. Montréal 2005
Modeling the dispersal of adult zooplankton from an isolated pond community.

Allen, M. Northeast Ecology and Evolution Conference. State College, PA 2005
Examining spatial and temporal heterogeneity in the dispersal of pond zooplankton.

Allen, M. & Shea, K. ESA Annual Meeting. Portland 2004
Spatial segregation of invasive *Carduus* thistle species in central Pennsylvania.

SERVICE AND LEADERSHIP

Graduate Students in Ecology and Evolutionary Biology, Pres., Treasurer 2007-2009
Oversaw the educational, social, financial and outreach components of GEEB student group.

MBLGTACC – 2008 Co-chair, Financial Officer, Oversight Committee 2006-2009
Co-chairperson for 3 day 1500 person conference for Midwestern LGBTA students featuring keynote speakers, workshops and entertainment. Fundraised $60K and oversaw $125K budget. Board member and first treasurer of national organization. Secured 501(c)3 status for organization.

Student Cultural Programming Fund Allocation Committee 2008-2009
Served on Dean of Students Office committee awarding funds to increase cultural awareness and understanding.

Graduate College Student Advisory Committee 2007-2009
Served on board providing advice and graduate student input to the Graduate College Dean.

LGBT Center Assistant Director Search Committee 2009

PEEC/GEEB Workshops Committee, Co-chairperson 2007-2008
Co-organized workshops on scientist-media relations and public policy for ecology and evolution students.

GEEB Graduate Symposium Committee, Chairperson 2006, Student Judge 2003-2009
Helped organize a one day symposium on graduate ecology and evolutionary research. As chair I oversaw the committee and presented welcoming remarks to speakers, recruits and other guests.

Volunteer at Ecological Society of America conference 2006, 2008
Conference mentors program, session presider

Salt Fork River Cleanup sponsored by Prairie Rivers Network 2003-2005

Reviewer for various journals: Ecography, Freshwater Biology, Global Change Biology, Hydrobiologia, Oecologia

MEMBERSHIPS

American Society of Limnology and Oceanography
Ecological Society of America
Graduate students in Ecology and Evolutionary Biology (GEEB)
Phi Beta Kappa – Lambda Chapter of Pennsylvania
Prairie Rivers Network
Society for the Study of Evolution

LGBT Center Assistant Director Search Committee 2009

PEEC/GEEB Workshops Committee, Co-chairperson 2007-2008
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